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Miguel A. Rodriguez-Bigas
Raul Cutait · Patrick M. Lynch
Ian Tomlinson · Hans F.A. Vasen *Editors*

Hereditary Colorectal Cancer

Springer

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Hereditary Colorectal Cancer

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Preface

Over the last quarter of a century, significant and explosive advancements have been made regarding the study of colorectal cancer. The wealth of information evolving is far reaching. From the first case report of the loss of the long arm of chromosome 5 in a patient with Familial Adenomatous Polyposis, to the cloning and identification of multiple genes involved in hereditary colorectal cancer, the field has progressed so we can now offer our patients genetic predisposition testing and better clinical management. Molecular mechanisms and the implications that some of these changes have for our patients is better understood. Rather than only discussing therapy these advances now allow us to discuss surgical prophylaxis and chemoprevention. Advances in the knowledge of familial colorectal cancer have not come easy and are due to the hard work of inquisitive investigators and clinicians, the development of advance instrumentations and molecular genetic techniques and most importantly from our patients and families. Without them we would not have been able to achieve this feat. Still, there is more to be done in the field. There are yet undiscovered syndromes, genes and molecular alterations which can and will change the lives of families and individuals. Thus, we cannot rely solely on what has been done, but need to continue to question existing research in the future.

The lack of a comprehensive reference book on hereditary colorectal cancer has been our driving force. The editors have gathered a multinational panel of experts to address the issues in *Hereditary Colorectal Cancer*. This book goes beyond the historical aspects of Familial Adenomatous Polyposis and the Lynch Syndrome. It further encompasses the basic and clinical aspects of less common and less understood syndromes such as the Hamartomatous Polyposis Syndromes and MutYH Associated Polyposis. An important section of *Hereditary Colorectal Cancer* is devoted to genetic counseling, an evolving area. In this section, several leading authorities describe the issues pertaining to genetic counseling around the world and within registries. Also addressed are the psychosocial aspects of hereditary colorectal cancer. This book will serve as a clinical reference, however, it will be also a useful guide for basic scientists, genetic counselors, and those interested in hereditary colorectal cancer.

While the book was being edited, one of our contributors and friend passed away. Jeremy Jass was the ultimate translational scientist. He was a pathologist and a basic

scientist whose contributions to the field are too numerous to state. The editors would like to express their gratitude for his contribution as well as for all his contributions to the advancement of understanding colorectal cancer. We also would like to express our most sincere appreciation to the editors at Springer who have been immensely helpful and patient with us. Lastly we have to mention our patients and our families whom without their support this project would have not been possible.

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Part I
History

Chapter 1
History: Familial Adenomatous Polyposis

Susan K. Clark, Kay F. Neale, and Robin K.S. Phillips

> *It would be difficult to find a more promising field for the exercise of cancer control than a polyposis family, because both diagnosis and treatment are possible in the precancerous stage and because the results of surgical treatment are excellent.*
>
> C.E. Dukes 1958

Abstract This chapter sets out to describe the developments leading to our current knowledge of familial adenomatous polyposis. An appreciation of historical context allows an enhanced understanding of contemporary paradigms and management of this condition.

Keywords Familial Adenomatous Polyposis • History • Colorectal Cancer

1.1 Why Is History Important?

Some are interested in history for its own sake, but for most of us its value lies in the way in which it explains the present and points the way forward. We hope that reading this chapter will give inspiration to those interested in learning more about this fascinating condition.

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1.2 Early Descriptions

A handful of descriptions of patients with multiple colorectal polyps were published in the late nineteenth century, some of which were undoubtedly cases of familial adenomatous polyposis (FAP) [1–4] although others were probably inflammatory pseudopolyps. Various different terms were used to describe the condition, including "disseminated polypi", "multiple adenomas", "multiple adenomatosis" and "multiple polyposis", but it does not seem to have been recognised as a distinct entity for some time. Two authoritative textbooks of colorectal surgery from that time, by Curling [5] of the London Hospital and Allingham [6] from St Mark's Hospital, go no further than to note that polyps can be multiple.

By 1901, a standard German text book [7] differentiated adenomatous polyposis clearly from sporadic adenomas and other types of polyps. In the following three decades, pathological classification [8] formalised the distinction between adenomatous polyps and inflammatory pseudopolyps, resulting in a well-described disease entity [9, 10], often known as "polyposis coli" or "polyposis intestini", defined by the macroscopic and microscopic appearances and inheritance.

Although Cripps' early description of the disease [2] was in a brother and sister, and others described three members of the same family [11] and a mother and child [12] with the condition, the Mendelian dominant mode of inheritance was defined by Cockayne [13] in 1927 (Fig. 1.1).

The observation that patients with this condition developed cancer [14] sparked interest in the relationship between adenomatous polyps and large bowel cancer. Lockhart-Mummery [15] recognised that it was the propensity to form polyps, and subsequently cancer, which was inherited, rather than the cancers themselves. He noted that polyps tend to appear in late childhood, and that death from multiple colorectal cancers at a young age is almost inevitable. He also commented on cases of colorectal cancer apparently developing from sporadic adenomas.

1.3 Foundation of Registries and Collaborative Groups

The St. Mark's Hospital Polyposis Register [later to become Registry in 1985] [16] was established in 1924 as a laboratory to examine the polyps taken from Lockhart-Mummery's first three families, the results of which were published the following year [15] (Fig. 1.2). The staff set about clarifying pedigrees and identifying at-risk family members, keeping meticulous records. Over the years, the role of the registry has expanded to include call-up, counselling, surveillance and genetic testing of at-risk relatives, provision of prophylactic surgery and recall for regular follow-up. The resulting database is an invaluable source of information, and the centralisation of care facilitates prospective research.

A number of other such registries have been developed around the world, but the first national register was established in Sweden [17]. There is evidence that patients

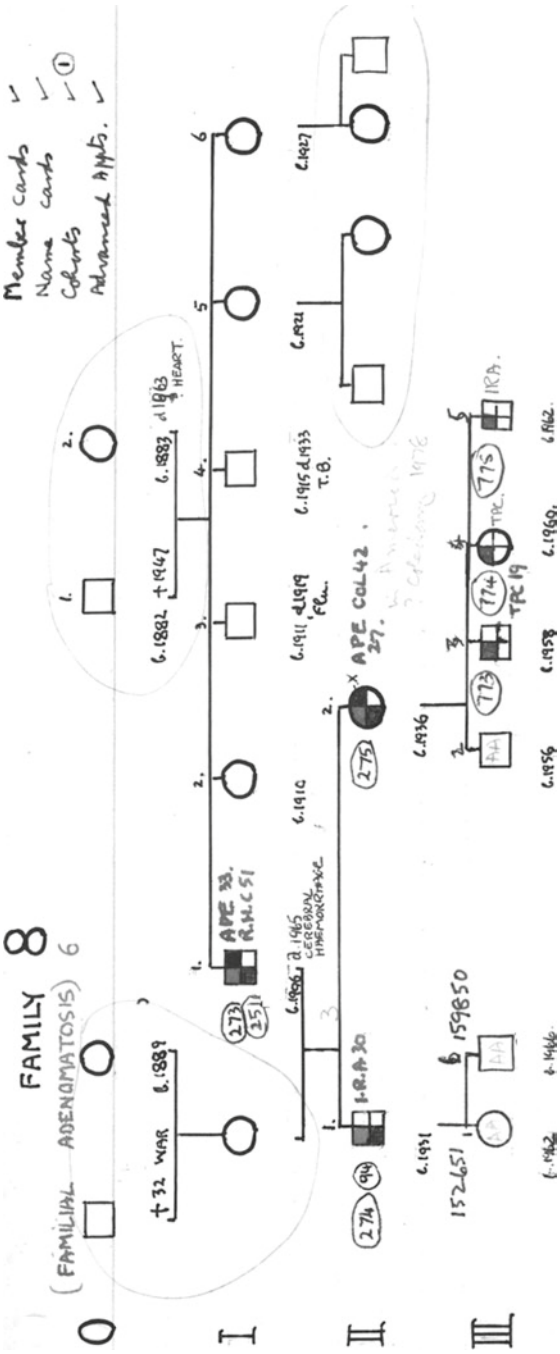


Fig. 1.1 An original family tree as drawn by Dr. HJR Bussey

Fig. 1.2 Dr. HJR Bussey in St Mark's Hospital Pathology Department 1992

with FAP cared for in a registry have a much lower chance of having colorectal cancer at the time of diagnosis of FAP [18] and higher life expectancy [19, 20] than those not cared for in such a setting.

1.3.1 The Leeds Castle Polyposis Group and International Society for Gastrointestinal Hereditary Tumours

In June 1985, the Leeds Castle Polyposis Group, an international group of clinicians from polyposis registries around the world, met for the first time [21]. The meeting was initiated by Ian Todd, a surgeon at St. Mark's Hospital, who despite many years of experience in caring for patients with FAP found that he did not know how to treat a young woman with a large desmoid tumour. The meeting agreed that an international group should be formed to promote understanding of the rarer manifestations of this uncommon condition. By 1992, 51 centres around the world were involved, and meetings were held every 2 years. In 1995, the group met jointly with the International Collaborative Group for HNPCC, and in 2003, the two organisations merged to form the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) (Fig. 1.3). As the Society's name suggests, it is an international, multidisciplinary, scientific organisation. Its mission is to improve the quality of care of patients and their families with any condition resulting in hereditary gastrointestinal tumours. More information can be found on the website (www.insight-group.org) with details regarding membership and the biennial scientific meetings (Table 1.1) (Fig. 1.4).

Fig. 1.3 The first meeting of InSiGHT, Newcastle, UK, 2005

Table 1.1 LCPG and Insight biennial scientific meetings

Year	Chairman	Location
1985	Ian Todd	Leeds Castle, Kent, UK
1987	Jerome DeCosse	Washington, USA
1989	James Thomson	Broadway, UK
1991	David Jagelman	Fort Lauderdale, USA
1993	Steffen Bülow	Copenhagen, Denmark
1995	Hartley Stern	Toronto, Canada
1997	Hans Vasen	Noordwijk, The Netherlands
1999	Finlay Macrae	Lorne, Australia
2001	Luccio Bertario	Venice, Italy
2003	James Church	Cleveland, USA
2005	John Burn	Newcastle, UK
2007	Takeo Iwama	Yokohama, Japan
2009	Gariela Moeslein	Dusseldorf, Germany
2011	Partrick M. Lynch Miguel A. Rodriguez-Bigas	San Antonio, USA
2013	Alan Spigelman Finlay Macrae	Melbourne, Australia

Fig. 1.4 The InSiGHT logo

1.4 Development of Clinical Understanding of FAP

Collection together of multiple families with FAP and an increasing awareness of polyposis in the medical community facilitated observation and documentation of the extra-colonic manifestations of the condition. Examples include the first description of congenital hypertrophy of the retinal pigment epithelium (CHRPE) in a patient with FAP in 1935 [22], who also had duodenal cancer, and the documentation of the co-existence of FAP and desmoid tumour [23]. Gardner's description [24] of individuals with FAP, epidermoid cysts, osteomas and desmoids was for a time thought to be a distinct syndrome, but since the identification of the APC gene this is no longer considered to be the case (Fig. 1.5). In 1983, Judith Kingston noted that children with hepatoblastoma were likely to have a parent with FAP [25] and 2 years later an increased risk of thyroid cancer was reported by Plail [26].

Refinement of understanding the phenotype has progressed hand-in-hand with advancing medical technology. In particular, gastroduodenoscopy [27] has allowed an appreciation of duodenal polyposis, and cross-sectional imaging has enabled visualisation of desmoids and adrenal adenomas [28]. As screening and prophylactic surgery have been increasingly employed, reducing early deaths from colorectal cancer, duodenal and periampullary cancers and desmoid disease have emerged as important causes of death [29] in patients with FAP.

1.5 FAP as a Model of Sporadic Colorectal Cancer

Dukes, Morson and Bussey [30] in the pathology department at St Mark's saw relatively large numbers of cases of FAP and of sporadic colorectal cancer. Their observations led to the description of the adenoma–carcinoma sequence [31, 32]

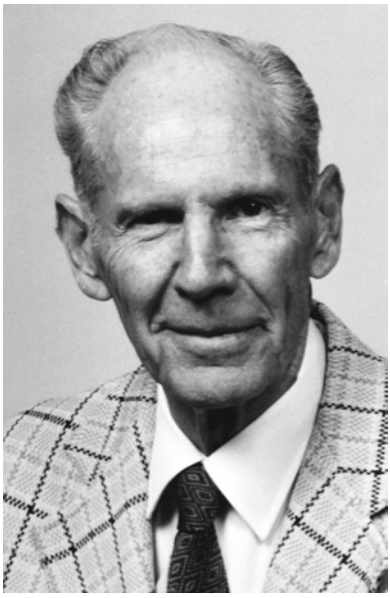


Fig. 1.5 Dr. Eldon Gardner

and the realisation that FAP can be viewed as a human model of colorectal cancer development. In 1968, Smith wrote that it would be "reasonable to hope that any significant discovery regarding familial multiple polyposis might have a bearing on the much larger problem of carcinoma of the colon" [33], and in 1975, Bussey expressed a similar view [30]: "it is the possibility of helping so many that justifies a study in depth of the few".

Much of the work underpinning Fearon and Vogelstein's proposed genetic pathway of colorectal cancer development [34] involved a study of the chromosomes and DNA from polyps removed from patients with FAP. Studies assessing potential chemopreventive agents for colorectal cancer have been undertaken on patients with FAP [35] who make an ideal model for this type of work.

1.6 Prophylactic Surgery

Lilienthal in North America performed the first recorded colectomy for FAP [36], the first operation in the UK being done on 18th March 1918 by JP Lockhart-Mummery at St Mark's Hospital [37]. The surgery was hazardous, and often done in stages. Rankin [38] described a three-stage proctocolectomy, in which an ileostomy was performed first, with a colectomy a few months later, and finally a perineal proctectomy. Mayo [39] reported a five-stage colectomy and ileosigmoidostomy done in two patients, one of whom died after the second stage from small bowel adhesions.

The introduction of prophylactic surgery followed an appreciation that pre-malignant adenomas inevitably progressed to invasive carcinoma, and an understanding of the inheritance of FAP provided the opportunity to identify asymptomatic patients in adolescence or early adulthood. While many patients diagnosed with FAP demanded that something be done, others either would not come for clinical assessment and screening or refused surgery [40].

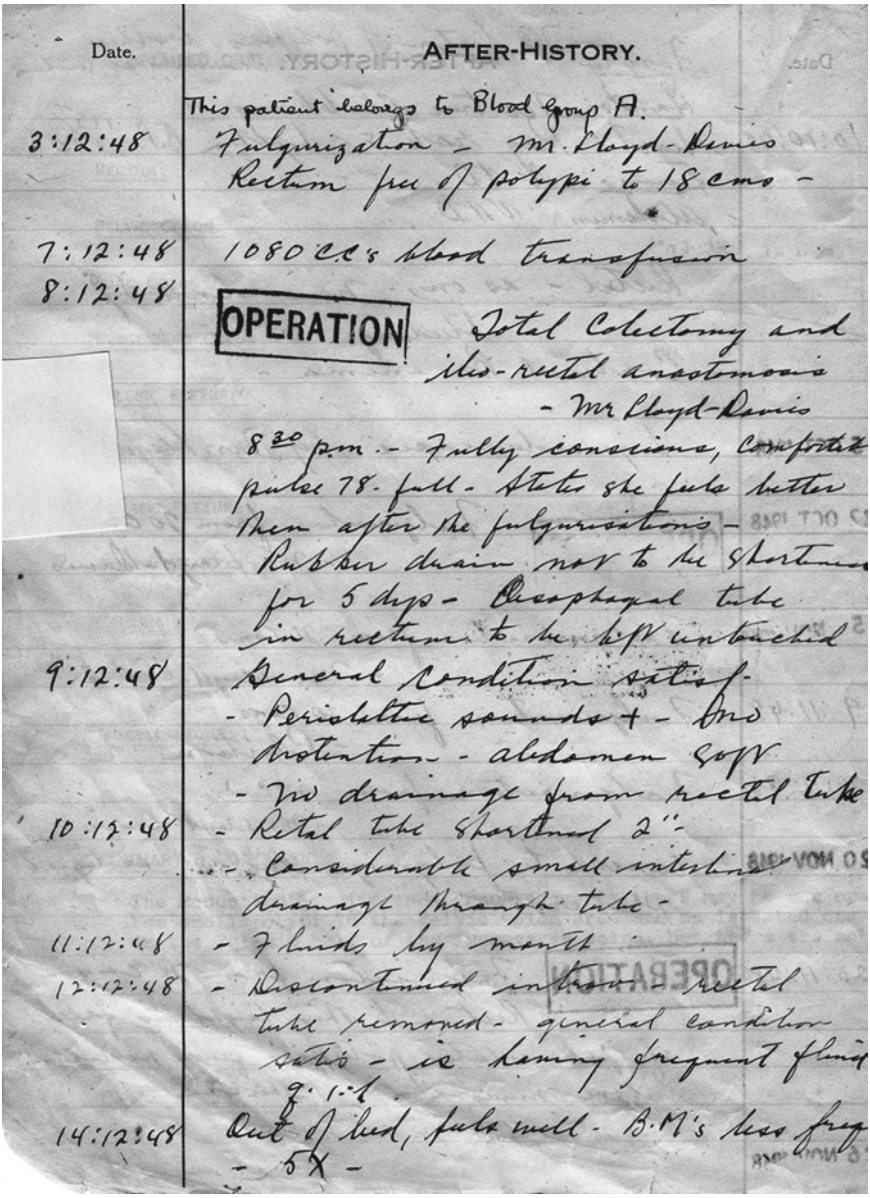
Gastrointestinal surgery was revolutionised in the 1940s by the development of antibiotics, blood transfusion and an understanding of the importance of electrolyte balance, all of which came at a time when anaesthesia was also becoming very much safer. The introduction of muscle relaxants (curare was first used in 1942) allowed much less anaesthetic agent to be used, decreasing cardiovascular depression. These advances allowed prophylactic single-stage colectomy with ileorectal anastomosis (IRA) to be performed relatively safely, with the great advantage of avoidance of an ileostomy. The first such procedure at St Mark's was carried out in 1948 by OV Lloyd-Davies [41] (Fig. 1.6).

1.6.1 Post-operative Follow-up

Patients after IRA required regular follow-up [42], done at that time with rigid sigmoidoscopy, much inferior to modern flexible endoscopes. In the early years, it was considered important to clear the rectum of polyps before colectomy and remove them on a regular basis afterwards. They were destroyed by fulguration [42], leading to considerable scarring, which made assessment of the state of the rectal mucosa more difficult in later years. Initial proctocolectomy or subsequent completion proctectomy was undertaken reluctantly, because of the resulting permanent ileostomy. While the risk of developing rectal cancer after IRA varies from series to series, probably in part due to different operative technique and follow-up protocols, the Mayo clinic reported a 59% risk after 23 years of follow-up [43], while at St Mark's [44], the equivalent figure was 10% by the age of 50 years and 29% at 60 years.

1.6.2 Advances in Surgical Technique

The advent of the ileoanal pouch, allowing restorative proctocolectomy (RPC) in the late 1970s [45] seemed to offer a solution to the problem of polyp and cancer development in the retained rectum after IRA. This procedure, however, is associated with greater morbidity than IRA, and a less satisfactory functional outcome [46]. There is a small risk to male sexual function, and a significant reduction in female fertility [47]. It is now becoming clear that adenomas and even carcinomas can develop in ileoanal pouches, which no longer seem to be the panacea they once did [48].


Date.

AFTER-HISTORY.

This patient belongs to Blood Group A.

3:12:48 Fulgurization – Mr. Lloyd-Davies
Rectum free of polypi to 18 cms –

7:12:48 1080 c.c.'s blood transfusion

8:12:48 OPERATION Total Colectomy and ileo-rectal anastomosis
– Mr Lloyd-Davies
8.30 p.m. – Fully conscious, comfortable
pulse 78. full – States she feels better
than after the fulgurisations –
Rubber drain not to be shortened
for 5 days – Oesophageal tube
in rectum to be kept untouched

9:12:48 General condition satisf.
– Peristaltic sounds + – bowel
distention – abdomen soft
– No drainage from rectal tube

10:12:48 – Rectal tube shortened 2" –
– Considerable small intestinal
drainage through tube –

11:12:48 – Fluids by mouth

12:12:48 – Discontinued intravenous – Rectal
tube removed – general condition
satis – is having frequent fluid
q. i. d.

14:12:48 Out of bed, feels well – B.M's less frequent
– 5X –

Fig. 1.6 Documentation from the patient's hospital record

1.6.3 Impact of Genetics on Surgical Choice

An understanding of the genotype–phenotype correlation allowing the identification of aggressive FAP, best managed by restorative proctocolectomy, and the advent of

flexible sigmoidoscopy and endoscopic polypectomy techniques mean that the rectal cancer risk after IRA is now much lower than it was in the "pre-pouch era" [49], and the pendulum is swinging back to IRA as the favoured prophylactic procedure in many cases. A further refinement is that IRA is increasingly being performed laparoscopically [50], a particularly attractive option for young people undergoing prophylactic surgery.

1.7 Gene Discovery

The structure of DNA was identified in the 1950s, but it was not until 1986 that the serendipitous observation of a deletion of the long arm of chromosome 5 in a patient with mental retardation and FAP led to the suggestion that the gene responsible would be found at that site [51]. In rapid succession, linkage studies confirmed this location [52] then refined it to 5q22. In 1991, causative mutations were identified in what was now called the APC gene [53] (the FAP gene having been named earlier as responsible for familial amyloidotic polypneuropathy) opening the door to predictive genetic testing, and more recently pre-implantation diagnosis, which is now available at a number of centres. As mutation detection methods improved it became possible to identify the mutations responsible for attenuated FAP allowing a genetic diagnosis to be made in cases not fulfilling the traditional clinical criteria of FAP.

Identification of a number of different mutations in the APC gene has allowed an understanding of genotype–phenotype correlation to be developed, although the mechanisms underlying this relationship remain to be fully elucidated. APC has also been found to have a pivotal role in the wnt signalling pathway, abnormal activation of which occurs in the majority of sporadic colorectal cancers.

1.7.1 MYH-associated Polyposis

Study of a small group of patients with a phenotype similar to attenuated FAP, but with no detectable APC mutation, and the fact that the handful of families with this condition apparently had recessive inheritance, led to the discovery of mutations in the MYH gene and identification of MYH-associated polyposis [MAP] [54].

1.8 History into the Future

History should be reviewed to confirm that the basic decisions regarding clinical care are still relevant to current clinical practise. For example, pioneers of the IRA would leave the recto-sigmoid long in an attempt to improve bowel function. Once

it had been shown that cancers could develop beyond the reach of a 25-cm rigid sigmoidoscope, most surgeons fashioned the anastomosis lower; with the advent of flexible scopes, there is an argument to return to the practice of the early pioneers.

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Chapter 2
Historical Aspects of Lynch Syndrome

Henry T. Lynch, Megan P. Hitchins, Trudy G. Shaw, Jane F. Lynch, and Hemant Roy

Abstract In 1895, Aldred Warthin, M.D., a pathologist with a keen interest in patients, and a good listener, noted that his seamstress appeared to be depressed. He pursued this in detail, and she told him it was because she believed she would die of cancer at an early age, since everyone in her family seemed to succumb to cancer of the colon or female organs. Just as she predicted, she developed endometrial cancer and died of that disease. This brief background piqued Warthin's interest and he developed her pedigree and many others. In a remarkably similar scenario, Henry Lynch, M.D., in 1962, while a first year internal medicine resident, was called to see a patient who was recovering from the delirium tremens. In a statement similar to that of Warthin's seamstress, the patient stated that he drank because he knew he would die of cancer since everyone in the family died of colorectal cancer. Lynch, assuming that he was dealing with familial adenomatous polyposis, developed the pedigree only to find that the colon cancers were occurring in the absence of multiple colonic adenomas. Other cancers, particularly the endometrium and ovary, occurred throughout the extended family, showing a pattern consonant with an autosomal dominant mode of genetic transmission. The syndrome in the family, along with a strikingly similar family, were published in the *Archives of Internal Medicine* in 1966. Since that report, many hundreds of hereditary cancer-prone families with the same patterns of cancer occurrences, now known as Lynch syndrome, have been identified throughout the world, and it is now the undisputed most common hereditary form of colorectal cancer. History shows that these discoveries were products of collecting detailed family histories and innovative reasoning concerning their clinical significance. This manuscript will show the historical development of our understanding about the importance of a comprehensive cancer family history involving cancer of all anatomic sites, genetic counseling, and DNA testing when indicated. The fervent hope continues that these practices will significantly reduce cancer's morbidity and mortality in the Lynch syndrome.

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2.1 Introduction

When one thinks of the clinical entity referred to as a "syndrome," the first questions that might come to mind are "How did it all start?" and "What criteria were used for a syndromy designation?" The syndrome pertinent to this chapter, namely the Lynch syndrome, had its beginnings in 1895 when Aldred Warthin, M.D., began his long tenure at the University of Michigan School of Medicine in Ann Arbor. At that time, his seamstress appeared depressed, and being an extremely inquisitive and caring physician, he asked her why she was depressed. She told him it was because she was convinced that she was going to die of cancer and that it would involve her gastrointestinal tract or her female organs, since "Everyone in the family dies of these cancers." This piqued Warthin's interest and he began compiling her pedigree, along with many others from the tumor registry at the University of Michigan; he found it to be quite alarming, since the very cancers that the seamstress had discussed with him were present through four generations. Also, just as she had predicted, she died at an early age of metastatic endometrial carcinoma. Warthin referred to the pedigree as Family G (Fig. 2.1) [1, 2].

In 1962, Lynch, then a 2nd-year resident in internal medicine, was called to see a patient who was recovering from delirium tremens and, in a statement remarkably similar to that of Warthin's seamstress, said that he knew he was going to die of colorectal cancer (CRC), which was highly prevalent in his family; he gave this as an excuse for his heavy drinking. Just as he had predicted, he died of cancer. At that time, the only known hereditary form of CRC was a syndrome known as familial adenomatous polyposis coli (FAP). When a working pedigree was initially developed in this patient's family, it was initially inferred that this most likely represented a form of FAP, given the large number of CRCs present throughout the family. However, this presumptive diagnosis changed significantly once pathology reports were secured, which uniformly showed no evidence of multiple colonic adenomas in any of the CRC affecteds. Indeed, evaluation of the pedigree showed a segregating pattern of not only CRC but, most remarkably, a plethora of extracolonic cancers, particularly carcinoma of the endometrium and the ovary, consonant with an autosomal dominant inheritance pattern. Some of the family members showed synchronous and/or metachronous CRCs. Some showed a striking pattern of both endometrial and colorectal carcinoma, while others manifested synchronous or metachronous endometrial and ovarian carcinoma. Meanwhile, Warthin's research on Family G had been buried in the literature, which made this new family (called Family N, designating its Nebraska origin) of enough interest that an abstract of the work was accepted by the American Society of Human Genetics for an oral

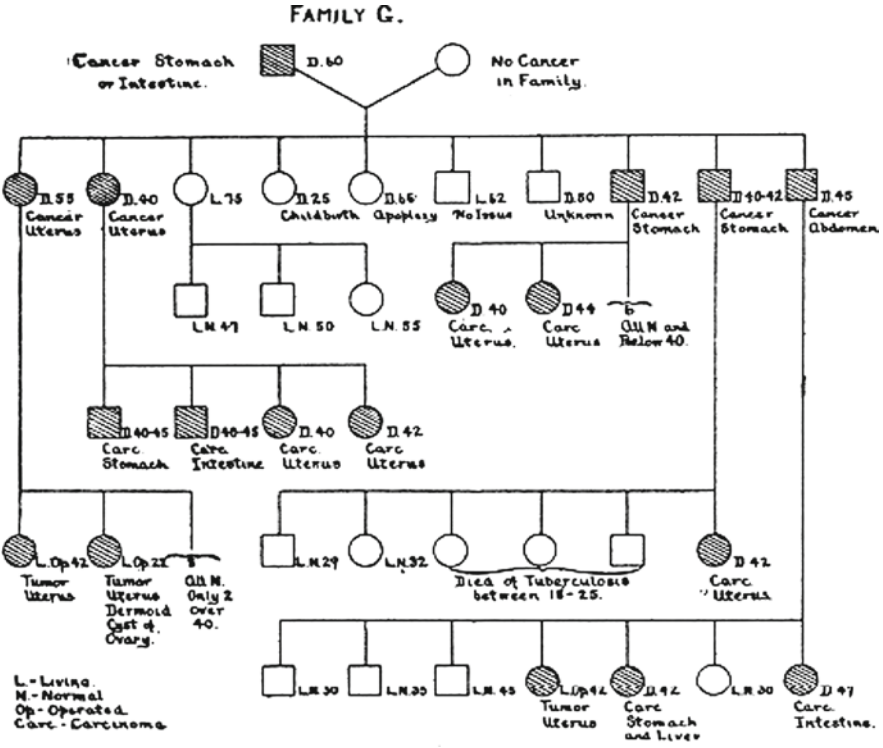
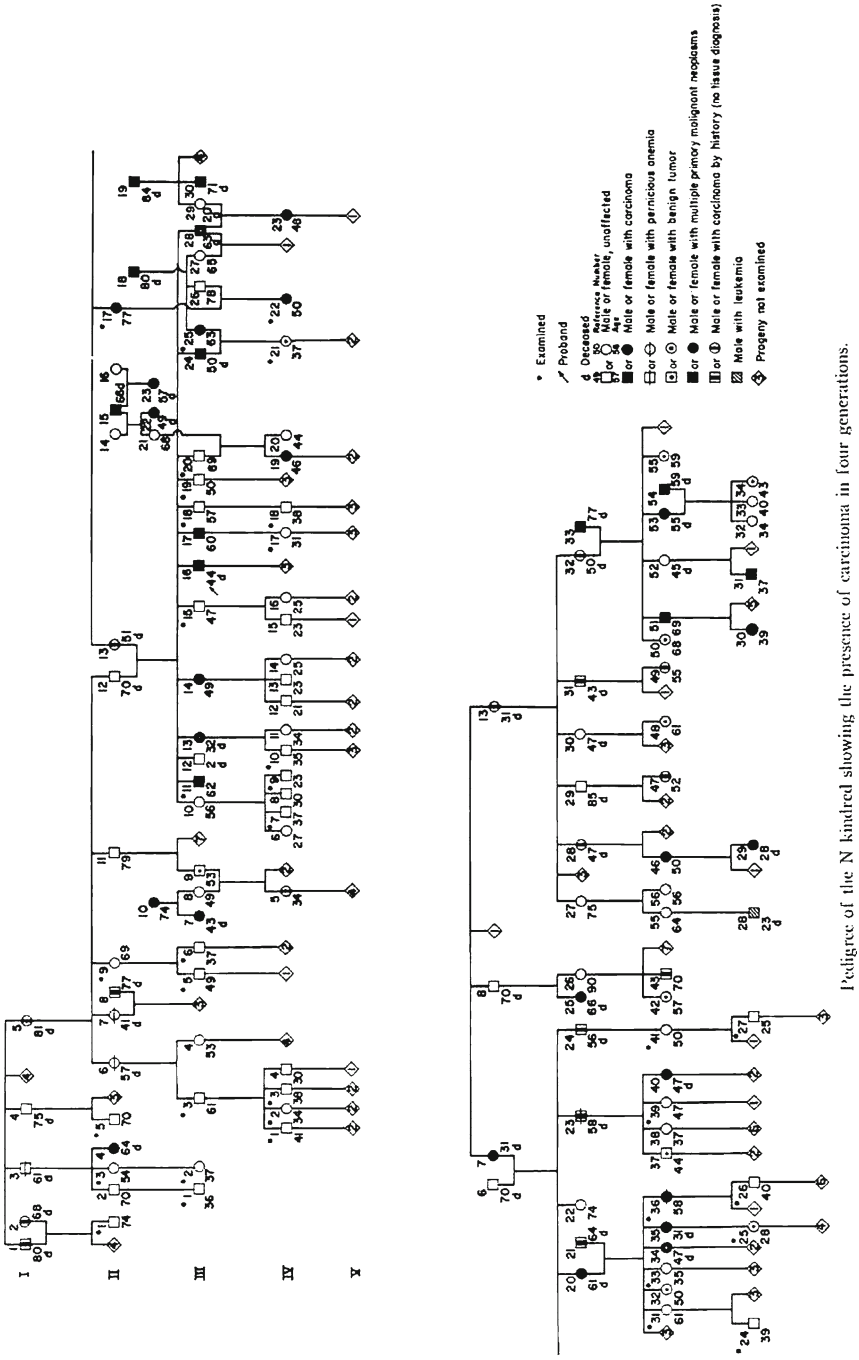


Fig. 2.1 Pedigree of family G

presentation in 1964. It was at that meeting that Marjorie Shaw, M.D., a medical geneticist at the University of Michigan, became intrigued with the report on Family N. She stated that she had a similar family (Family M, designating Michigan) and would like to collaborate with Lynch. This led to the publication of both families in 1966 (See Figs. 2.2 and 2.3) [3].

As a result of the reports on these families, an invitation from A.J. French, M.D., Warthin's successor as Chairman of Pathology at the University of Michigan, offered Lynch access to all of the pedigree scrolls, slides and tissue blocks that Warthin had meticulously compiled and which were, as Dr. French stated, "simply gathering dust in a closet in my office." Lynch and a social worker (Ann Krush, M.S.W.) pored over the records during multiple trips to Ann Arbor, had re-reviews of the pathology performed by a pathology colleague (Arthur Larsen, M.D.), and made several "field visits" to descendants of Warthin's Family G who resided throughout the Ann Arbor region, in order to help update the pedigree.

Lynch and Krush then traveled to the region of Germany from which Family G originated and from where family members had emigrated to the United States, settling in the region surrounding Ann Arbor, Michigan. Many relatives still resided in farming communities south of Stuttgart. Lynch and Krush engaged many family



Pedigree of the N kindred showing the presence of carcinoma in four generations.

Arch Intern Med—Vol 117, Feb 1966

Fig. 2.2 Pedigree of family N

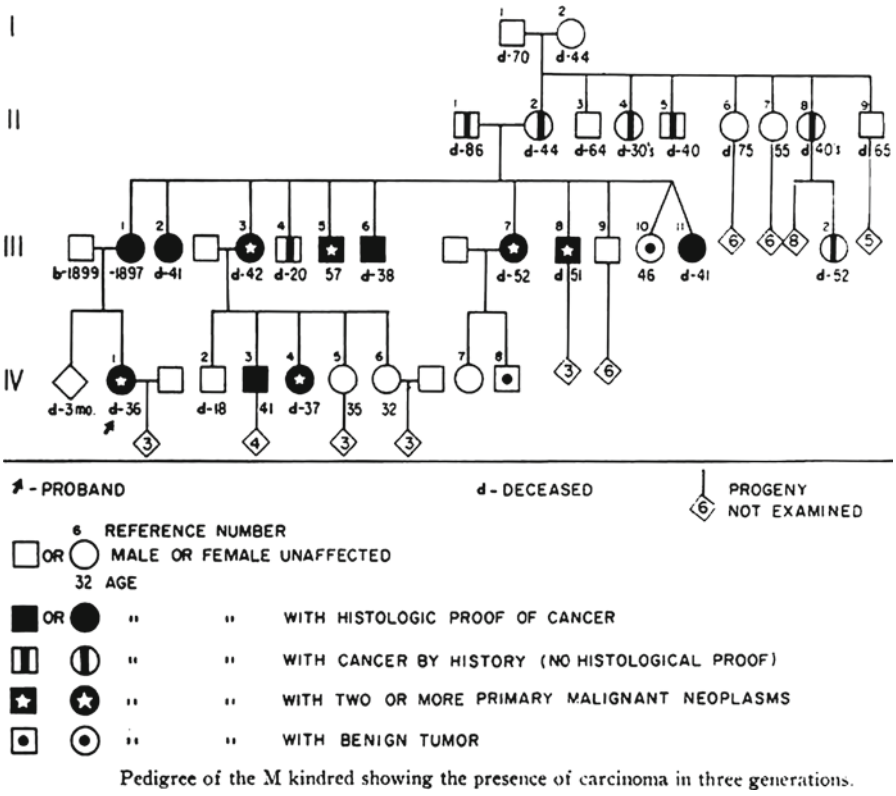


Fig. 2.3 Pedigree of family M

members and continued their intensive search for information that was relevant to the origins of cancer in the family and that, thereby, could be incorporated into the pedigree. Whenever possible, pathology verification of cancer was obtained. An update of Family G was then published [4]. Advances in genetic testing subsequently made it possible to identify Family G's specific causal mutation in the year 2000 [5], slightly more than a century after Warthin's seamstress first told him about her cancer family history. Family G is still being followed by Lynch and colleagues at Creighton University and at the University of Michigan, with the most recent update of this family appearing in 2005 [6].

Because of the lack of multiple colonic adenomas, acceptance of these "cancer families" as evidence of a valid hereditary cancer syndrome was exceedingly low. Indeed, prior to advances in molecular genetics, culminating in the discovery of causal mutations in the mismatch repair (MMR) genes [7–9], the etiology of these familial cancer aggregations was considered to be a function of chance and/or environmental causes (some suggested the latter because of the families' farming backgrounds in the Midwest). This reasoning made approval for funding extremely difficult. Nevertheless, because of the clear Mendelian inheritance pattern of

cancers in these families, Lynch continued to investigate what he strongly believed to be a primary genetic etiology as causal for the cancer aggregations, by using his own out-of-pocket funds. For lack of a better term, Lynch referred to Families N, M, and G, as "cancer families." The designation was then changed to hereditary nonpolyposis colorectal cancer (HNPCC), which actually turned out to be a poor choice, given the fact that occasional polyps were present in CRC affecteds and that the mentioned litany of extracolonic cancers required an explanation [10]. The disorder was subsequently referred to as Lynch syndrome [11, 12].

As more families manifesting the cancer family syndrome were identified, Lynch and his associates developed the Family Information Service (FIS) [13], which evolved through the experience of visiting families throughout the Midwest using a customized recreational vehicle (RV) which had an interview room, examining room, and a laboratory for blood draws. This enabled them to go directly to those geographic areas where numbers of family members resided and to educate both high-risk patients and their family physicians about the genetics, natural history, surveillance, and management of the Lynch syndrome. An important aim of this approach was to gather data to support the contention that the syndrome was a *bona fide* clinical phenomenon even though its existence was being severely challenged. As the number of interested family members began to increase markedly, it became necessary to meet with families in surroundings much larger than those available in the RV, and FISs are now usually held in meeting rooms of local hospitals, or in the office of a physician who is involved in the health care of members of the family.

2.2 Discovery of Lynch Syndrome's Natural History

Table 2.1 tracks the development of the syndrome's history, dating back to Family G, and then, with greater force, Families N and M, and others from the late 1960s to the present. Not unexpectedly, throughout that time new clinical, pathologic, and molecular genetic discoveries emerged which collectively further elucidated the syndrome's clinical and molecular genetic etiology. Perhaps one of the most important aspects of its "cardinal features" was the strikingly early age of cancer onset, which is approximately 20–25 years earlier for its integral syndrome-related cancers when compared with their sporadic counterparts. For example, the average age of onset for CRC in Lynch syndrome is approximately 44 years. This clinical finding was followed by the discovery of a proclivity to proximal colonic cancer [14] and an excess of synchronous and metachronous cancer occurrences, including an excess of multiple primary cancers, the most common of which were found to be CRC and endometrial carcinoma.

In the late 1980s, criteria for Lynch syndrome began to emerge. The first set was referred to as the Amsterdam Criteria [15] and was intended to be used to assure that collaborating researchers in different parts of the world were following common criteria in classifying research subjects, as opposed to being used to diagnose the syndrome clinically. However, the Amsterdam Criteria were found to be rather

Table 2.1 Landmarks of lynch syndrome history

Feature	First report	References
Family G of Warthin (study began 1895)	1913	[1]
Genetic counseling	1965	[69, 70]
First report of Lynch et al. on families N and M	1966	[3]
Early age of cancer onset	1966	[3]
Autosomal dominant inheritance pattern	1966	[3]
Family information session (FIS)	1966	[3, 13, 71]
Screening recommendations	1967	[72]
Update of family G	1971	[4]
Proximal colon involvement	1977	[14]
Beginning of study of Lynch syndrome in Uruguay	1977	[73]
Recommendation of prophylactic TAH-BSO	1978	[74]
Muir–Torre syndrome (as variant of Lynch syndrome)	1980	[26, 75]
Increased incidence of synchronous and metachronous CRC	1982	[76, 77]
Lynch syndrome studies begin with the Navajo	1983	[9, 78–82]
Tritiated thymidine distribution studies of rectal mucosa	1983	[83]
HNPCC named "Lynch syndrome"	1984	[11]
Selenium levels in Lynch syndrome studied	1984	[84]
Formation of ICG-HNPCC	1989	[15, 85]
Lectin binding studies in FAP and HNPCC	1990	[86–88]
Amsterdam I criteria	1991	[15]
Accelerated carcinogenesis and interval CRC	1992	[22, 89–92]
First cancer susceptibility locus found on 2p through linkage analysis	1993	[7]
Second cancer susceptibility locus found on 3p through linkage analysis	1993	[8]
DNA mismatch repair genes reported	1993	[19, 31, 32, 93]
RER+ (MSI) phenotype described	1993	[94]
Germline mutations in the syndrome	1993	[93]
MSH2 mutation identified	1993	[19]
Extracolonic adenocarcinomas	1994	[95]
Distinctive pathology features	1994	[90]
MSH2; *MLH1* mutations identified	1994	[31, 32]
PMS2 mutations identified	1994	[33]
Creighton group's involvement in Uruguayan study	1995	[96]
Historical perspective through 1995	1995	[97]
Role of DNA MMR genes in CRC tumorigenesis	1995	[98, 99]
Recommendations of prophylactic subtotal colectomy	1996	[65, 100]
Survival advantage	1996	[30, 101]
NIH NCI workshop on HNPCC (Bethesda Guidelines)	1996	[102]
MSH6 mutation	1997	[34, 103]
NIH NCI update on MSI	1997	[17]
Small bowel involvement	1998	[104]
Founder mutation in Finland	1998	[105]
Amsterdam II criteria	1999	[16]

(continued)

Table 2.1 (continued)

Feature	First report	References
Tumor-infiltrating lymphocytes and their association with MSI	1999	[20]
Conversion technology	2000	[5]
Development of light scattering to probe epithelial architecture	2000	[55]
A complex mutation of *MLH1* at codon 222 is associated with adolescent onset of CRC (more early onset CRC families needed for study)	2001	[106]
Germline epimutation of *MLH1* gene	2002	[45]
Fluorouracil-based adjuvant chemotherapy benefits patients with stage II or stage III CRC with MSS or MSI-L tumors but not those with MSI-H tumors	2003	[107]
H(2)O(2) effect improves survival in DNA MMR-deficient cell line	2003	[108]
MSH2 del1-6 founder mutation in the United States	2003	[109]
Amsterdam criteria positive families without evidence of MMR mutations	2005	[40]
Later age of cancer onset determined for Lynch syndrome cancers	2005	[110]
Lynch syndrome with extremely early-onset CRC, hematological malignancies, and neurofibromatosis features	2005	[111]
Enhanced backscattering spectroscopy analysis of the uninvolved colonic mucosa used to stratify risk of CRC	2006	[59]
Familial epimutation of *MSH2* described	2006	[53]
Description of an inherited germline epimutation of *MLH1* gene	2007	[49]

stringent, so that a less stringent version was developed and was referred to as Amsterdam Criteria II [16]. Following this, an even more widely-embracing approach to diagnosis of the syndrome was developed, referred to initially as the Bethesda Guidelines [17]. These guidelines were subsequently expanded by including pathology features, and since then have been known as the Revised Bethesda Guidelines [18] (See Table 2.2).

Discovery of the mentioned MMR mutations (*MSH2*, *MLH1*, *MSH6*, *MLH3*, *PMS2*), which began to be described in the early and mid-1990s [7, 8, 19], enabled the identification of mutation carriers, which then provided the level of certainty needed to "clinch" the concept that "cancer families" did, indeed, represent a specific hereditary cancer syndrome. Other important natural history findings began rapidly emerging, particularly certain distinguishing pathology features [20, 21], characterized by poorly differentiated CRCs with mucoid features, signet cell excess, peritumoral lymphocytic infiltration, Crohn's-like reaction, increased lymphocytic infiltration, and accelerated carcinogenesis. The combined discoveries of excess proximal CRCs [14] and accelerated carcinogenesis [20–22] impacted

Table 2.2 Amsterdam I and Amsterdam II Criteria, and Bethesda Guidelines

Amsterdam I criteria [15]
At least three relatives with histologically verified colorectal cancer
One is a first-degree relative of the other two
At least two successive generations affected
At least one of the relatives with colorectal cancer diagnosed at <50 years of age
Familial adenomatous polyposis has been excluded
Amsterdam II criteria [16]
At least three relatives with an hereditary nonpolyposis colorectal cancer-associated cancer (colorectal cancer, endometrial, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin [sebaceous tumors])
One is a first-degree relative of the other two
At least two successive generations affected
At least one of the hereditary nonpolyposis colorectal cancer-associated cancers should be diagnosed at <50 years. of age
Familial adenomatous polyposis should be excluded in any colorectal cancer cases
Tumors should be verified whenever possible
Bethesda Guidelines for testing of colorectal tumors for microsatellite instability [18]
Colorectal cancer diagnosed in a patient who is less than 50 years of age
Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors,[a] regardless of age
Colorectal cancer with the MSI-H[b] histology[c] diagnosed in a patient who is less than 60 years of age[d]
Colorectal cancer or HNPCC-associated tumor[a] diagnosed under age 50 years in at least one first-degree relative[e]
Colorectal cancer or HNPCC-associated tumor[a] diagnosed at any age in two first- or second-degree relatives[e]

[a]Hereditary nonpolyposis colorectal cancer (HNPCC)-associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter or renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas, and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel

[b]MSI-H = microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers

[c]Presence of tumor infiltrating lymphocytes, Crohn disease-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern

[d]There was no consensus among the workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines

[e]Criteria 4 and 5 have been reworded to clarify the Revised Bethesda Guidelines

surveillance and management strategies, culminating in the recommendation for initiation of annual colonoscopy at age 25 [23].

The Muir–Torre syndrome [24, 25], characterized by sebaceous adenomas/carcinomas and multiple keratoacanthomas, which is now known as a form of Lynch syndrome, was first described by Lynch et al. [26, 27] and subsequently updated [28, 29]. Figure 2.4 is the pedigree of a Muir–Torre family that also manifests a wide variety of extracolonic cancers integral to the Lynch syndrome.

Extracolonic malignancies were identified as being integral to the Lynch syndrome; the most prominent of these are the mentioned endometrial carcinoma

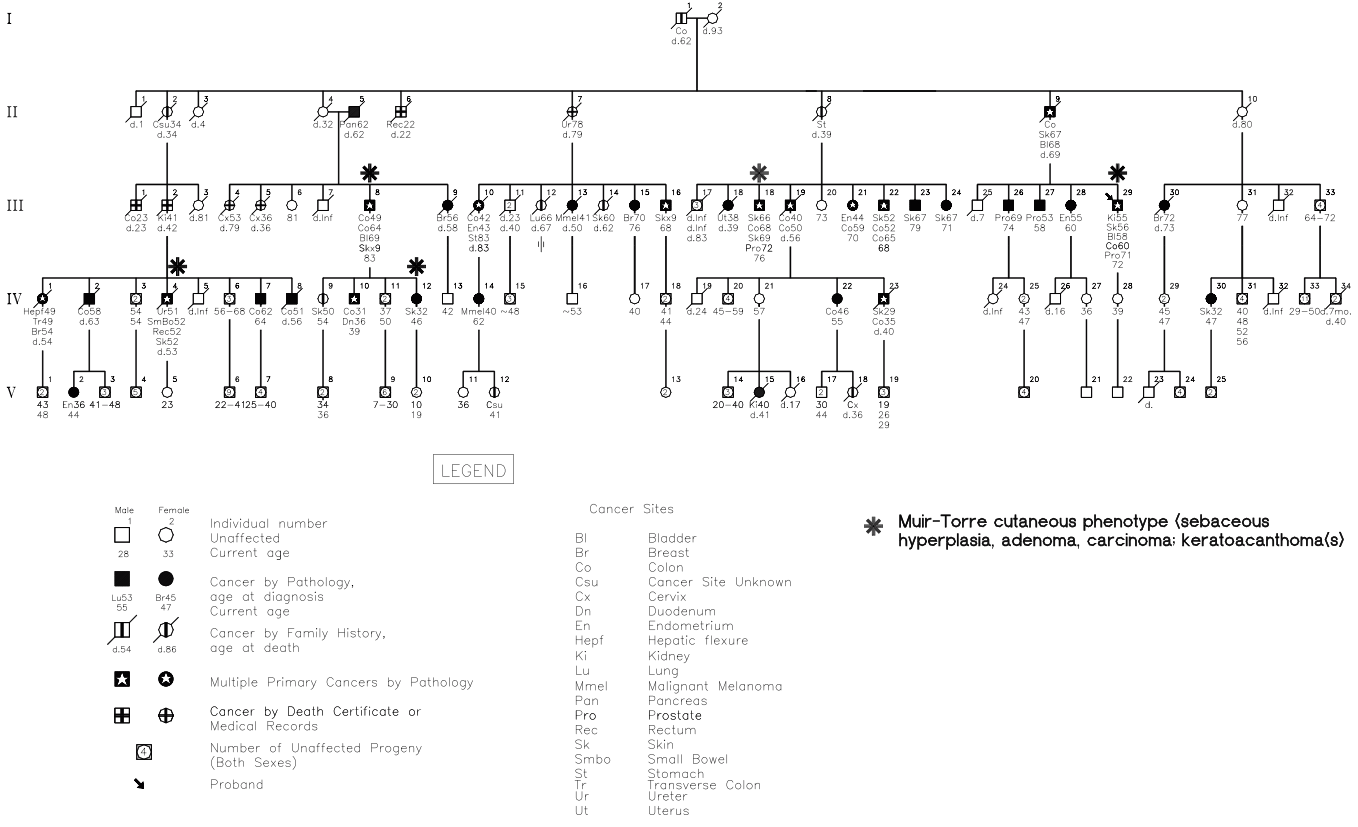


Fig. 2.4 Pedigree of a family with the Muir–Torre variant of Lynch syndrome, manifesting a variety of cancers integral to the syndrome

which occurs in about 60% of female mutation carriers, and ovarian cancer which occurs in about 12%. Other integral cancers include upper uro-epithelial tract (ureter and renal pelvis) transitional cell carcinoma in about 4% of mutation carriers, carcinoma of the stomach in 13% (particularly in Orientals indigenous to Japan and Korea), cancer of the small intestine in 5%, hepatobiliary tract in about 2%, and brain tumors in about 4%, with CRC being the most prominent, occurring in about 82%. These figures vary with differing ethnic and racial groups.

An interesting conundrum in the Lynch syndrome is its survival advantage [30]. Specifically, in a study of 274 cases (from 98 Lynch syndrome families) and 820 case-consecutive CRC series, it was found that cases were lower stage at diagnosis than controls. This may have been due to rare distant metastases at diagnosis. In these cohorts, the estimated death rates in Lynch syndrome cases, when adjusted for age and stage differences, was at most only two-thirds of the controls [30]. This survival advantage has led to the hypothesis that mutator genes (*MLH1*, *MSH2*) cause genomic instability, which poses an increased burden of microsatellite disturbance that in turn overwhelms CRC, sending cells to apoptosis. An immune response, particularly tumor infiltrating lymphocytes admixed with the tumor cells, may also be contributory to this better survival [20].

2.3 1990s: Discovery of Mutations of the Mismatch Repair Genes in Lynch Syndrome

Identification of the components of the mismatch repair system by Kolodner and colleagues in the late 1980s–early 1990s was soon followed by the identification of loss-of-function mutations of the mismatch repair genes in Lynch syndrome families; *MSH2* in 1993, *MLH1* and *PMS2* in 1994, and *MSH6* in 1997 [19, 31–34]. While variants within the *PMS1* gene have been identified [33], their causal role in Lynch syndrome remains to be proven, since no definitive pathogenic mutations of this gene have been identified that segregate with the phenotype in affected families [35].

Mutations of *MSH2* and *MLH1* are most prevalent in Lynch syndrome, each accounting for approximately one third of all cases, whereas mutations of the remaining mismatch repair genes collectively account for a small percentage of cases. However, the paucity of *PMS2* mutations identified may reflect the difficulty posed in screening this gene due to the existence of multiple pseudogenes with strong sequence homology [36]. Germline mutations of the mismatch repair genes are classically heterozygous, and tumor development conforms to Knudson's "two-hit" hypothesis whereby the germline mutation serves as the "first hit" in conferring an inborn susceptibility to cancer, followed by loss of function of the wild-type allele in the vulnerable somatic tissues, usually through an acquired deletion or point mutation [37]. Databases of the multiplicity of disease-causing mutations and missense variants of unknown pathogenicity within the various mismatch repair genes in Lynch syndrome have now been compiled, and are publicly available

through the world wide web and updated on a regular basis [38]. The identification of the genes responsible for the major proportion of Lynch syndrome patients has had an enormous impact on diagnosis, genetic counseling, and the clinical management of patients.

Further study of the Lynch syndrome showed that the clinical cancer phenotypes appear to differ in relation to which MMR gene is mutated in the family. Specifically, *MSH2* mutations have an increased frequency of extracolonic cancer types and more commonly show the Muir–Torre syndrome features. *MLH1* mutations appear to have an increased CRC expression with a slightly lower frequency of extracolonic cancer expression. Mutations in *MSH6* appear to be more "benign" with a decrease in CRC but an excess of endometrial carcinoma. It has been suggested that *PMS2* mutations give rise to a milder phenotype, with a more advanced age of onset in cases with heterozygous mutations, and biallelic mutations have been described in rare instances of the recessive Turcot syndrome in which brain tumors occur concurrently with colorectal tumors [39]. Now that these features convincingly herald the Lynch syndrome, we are now finding variants of Lynch syndrome-like families. Specifically, Lindor et al. [40]. described families that fulfilled the Amsterdam criteria but lacked MMR mutations. These families, while mimicking Lynch syndrome, nevertheless appear to be more benign with a lesser frequency of CRC and extracolonic cancers, and a generally later age of onset. A group of investigators in Spain have reported similar findings [41].

2.4 Microsatellite Instability and Immunohistochemistry Testing

Microsatellite instability (MSI) and immunohistochemistry (IHC) have proven to be extremely helpful in the diagnosis of Lynch syndrome [42]. MSI is a mutation signature in CRCs that evolve through inactivation of the DNA MMR system, giving rise to altered lengths of tandem repeat units within microsatellite sequences in the tumor DNA, and is found in approximately 15% of all CRCs. Approximately 3% of all CRCs arise from Lynch syndrome and nearly all Lynch syndrome CRCs are MSI^+ [43]. Twelve percent of CRCs represent non-inherited forms of DNA MMR inactivation, and in the late 1990s, these were found to be induced by methylation of the promoter of both copies of the *MLH1* gene, which silences gene expression [44]. These sporadic MSI^+ cancers arise most frequently in older individuals, and since the promoter methylation in these cases is acquired in the somatic tissues and essentially confined to the tumor, they are sometimes referred to as "somatic epimutations." MSI provides clinical information in the evaluation of a subset of CRC patients and is within the grasp of molecular diagnostic laboratories. Interestingly, discovery of MSI in CRC showed patients with MSI^+ tumors to have better rates of survival, particularly in younger patients. The application of IHC has been crucial in indicating which of the various MMR proteins is responsible for tumor development in patients with Lynch syndrome, and therefore which

gene is likely to harbor a germline mutation prior to genetic screening. Yet despite the completion of the human genome sequence in 2003 and significant advances in genetic screening technologies, no further candidate genes have been identified and sequence mutations of the known mismatch repair genes still fail to account for up to one third of Lynch syndrome cases.

2.5 Germline Epimutations as a Cause for Lynch Syndrome

Recently, attention has been drawn to the role of epigenetic alterations arising in the germ line, so-called "germline epimutations," as a cause for Lynch syndrome. "Germline epimutation" represents a novel mechanism for disease in which the affected allele of a gene is rendered silent in the germ line by an epigenetic aberration. Such epimutations of the *MLH1* and *MSH2* genes have recently been identified in a small number of patients presenting with a clinical phenotype consistent with Lynch syndrome.

2.5.1 2002: Identification of Germline Epimutation of MLH1

The first case of a germline epimutation of *MLH1* was identified in 2002 with the finding of methylation of a single allele of the *MLH1* promoter in the peripheral blood, and deletion of the normal unmethylated allele in the tumor, in a sporadic case with Lynch syndrome [45]. A clearer picture of the role and etiology of this defect in Lynch syndrome came with the identification of additional cases (Table 2.3) [45–50]. These individuals have dense methylation of the large CpG island spanning the *MLH1* promoter on a single genetic allele throughout their normal somatic tissues, indicating the defect originated in the germ line (Fig. 2.5a) [47–49]. Germline epimutations result in the affected allele becoming switched off in the somatic tissues for the duration of the individual's life (Fig. 2.5b) [48, 49]. Cases with germline epimutations of *MLH1* are distinguishable from the more frequent sporadically arising MSI^+ colorectal cancers in older individuals as the latter have localized biallelic *MLH1* methylation (essentially confined to the tumor) (Fig. 2.5a).

Germline epimutations have typically been identified through the detection of methylation at the *MLH1* promoter in DNA extracted from normal somatic tissues such as peripheral blood. This defect occurs in the absence of sequence mutations within the *MLH1* locus and hence is not identifiable by conventional genetic screening techniques. There are various molecular techniques for the detection of CpG methylation, but each is based on the prior treatment of the genomic DNA sample with sodium bisulphite, which in turn serves as the template for subsequent PCR amplification [51]. In this treatment, unmethylated cytosines within the DNA react with the sodium bisulphite and are converted to thymines (via uracil) following PCR amplification. Conversely, methylated cytosines at CpG dinucleotides are

Table 2.3 Clinical phenotype of individuals with germline *MLH1* epimutations

Case	Sex	Primary cancer	Age (year)	Family history	References
Case 1	F	Colon	25	None	[45]
H166	F	Colon (ascending)	38	None	[46]
H403	M	Colon (transverse)	28	No FDR	[46]
H450	F	Colon (ascending)	23	No FDR	[46]
H628	M	Colon (descending)	17	No FDR	[46]
		Colon (ascending)	29		
VT	F	Cecum	46	Mother	[47]
		Endometrium	53	Colon; 64 years	
		Melanoma	57		
		Breast	63		
TT	M	Cecum	43	Mother	[47]
		Colon (descending)	44	Endometrium; 55 years	
		Duodenum	51		
		Ampulla of Vater	59		
ST	M	Colorectum	39	None	[48]
Case 2	M	Epidermoid lip	34	No FDR	[50]
		Cecum	35		
Patient A	F	Endometrium	45	None, transmitted to one of three sons	[49]
		Colon	59		
		Rectum	60		
Patient B	F	Colon	41	None	[49]
		Rectum	45		

The gender (*F* female, *M* male), site of the primary carcinomas, age of diagnosis in years (y), and family history (*FDR* no first-degree relatives) are given

unreactive, and remain as cytosines. Methylated DNA, which has retained its "CpG" dinucleotides, may thus be differentiated from unmethylated DNA in which the corresponding sites have been converted to "TpG," using any method that exploits the nucleotide differences (Fig. 2.6a). The simplest of these techniques is COBRA (*co*mbined *b*isulphite and *r*estriction *a*nalysis), which distinguishes methylated DNA on the basis of a restriction digest, in which the enzyme recognition site contains a CpG within the amplified fragment (Fig. 2.6b) [52]. Alternatively, cloning and sequencing of individual alleles following PCR amplification allows the patterns of allelic methylation to be determined in cases heterozygous for a SNP within the promoter fragment (Fig. 2.6c).

2.5.2 Phenotypic Features

Since individuals with germline *MLH1* epimutations bear only one functional copy of the gene, they have an equivalent life-time risk of developing Lynch syndrome-type cancers as their counterparts with heterozygous germline

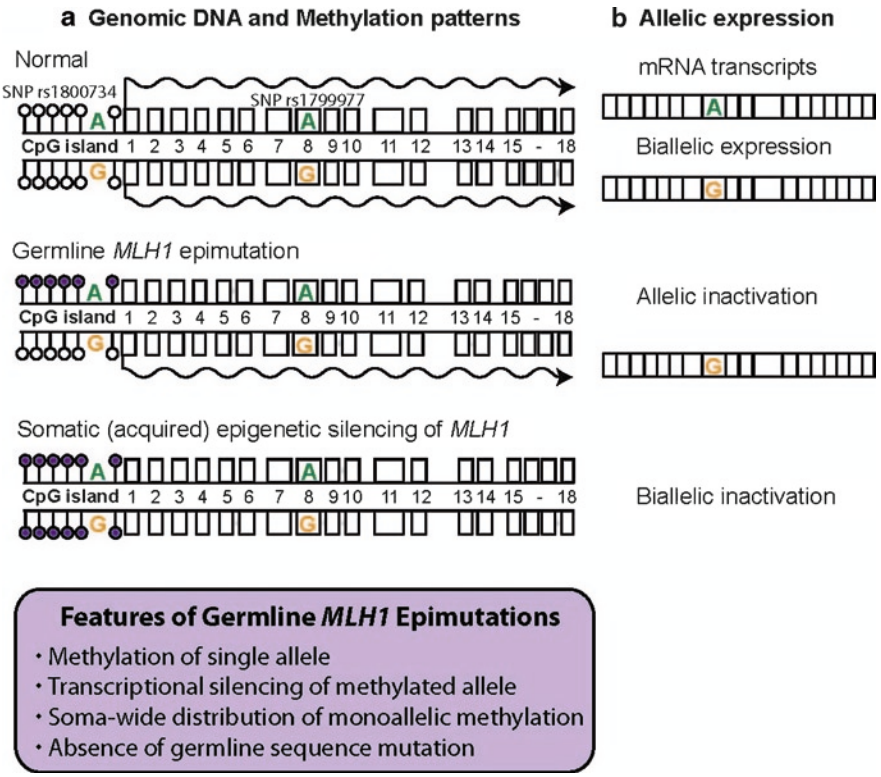


Fig. 2.5 Characteristics of germline *MLH1* epimutations: Illustration of the features of germline *MLH1* epimutations as compared with the normal state and sporadic microsatellite unstable colorectal cancers due to somatic *MLH1* methylation in older individuals. (**a**) Illustration of the *MLH1* gene. The CpG island is shown as lollipops, with methylated CpG dinucleotides colored *purple* and unmethylated CpGs in *white*. Exons are depicted as *boxes and numbered* (not strictly to scale). Transcriptional activity is denoted by the *waved arrow*. G/A SNP sites that distinguish the two genetic alleles of *MLH1* are shown according to their positions and labeled with their identifier. (**b**) Allelic transcription of *MLH1* is depicted as joined exons, with expressed alleles differentiated by the exonic G/A SNP. A single allele of *MLH1* is expressed in the somatic tissues in cases with a germline epimutation

sequence mutations. Individuals with germline *MLH1* epimutations typically develop cancers of the colorectum and endometrium at an early age (<50 years). The tumors demonstrate both MSI positivity and immunohistochemical loss of MLH1 protein expression [45–48]. Approximately half of the patients have developed multiple metachronous cancers, and some have additionally developed carcinomas atypical of Lynch syndrome, suggesting this defect may confer a particularly severe phenotype, though the full clinical spectrum associated with germline *MLH1* epimutations will be delineated as more patients are identified (Table 2.3) [47, 50].

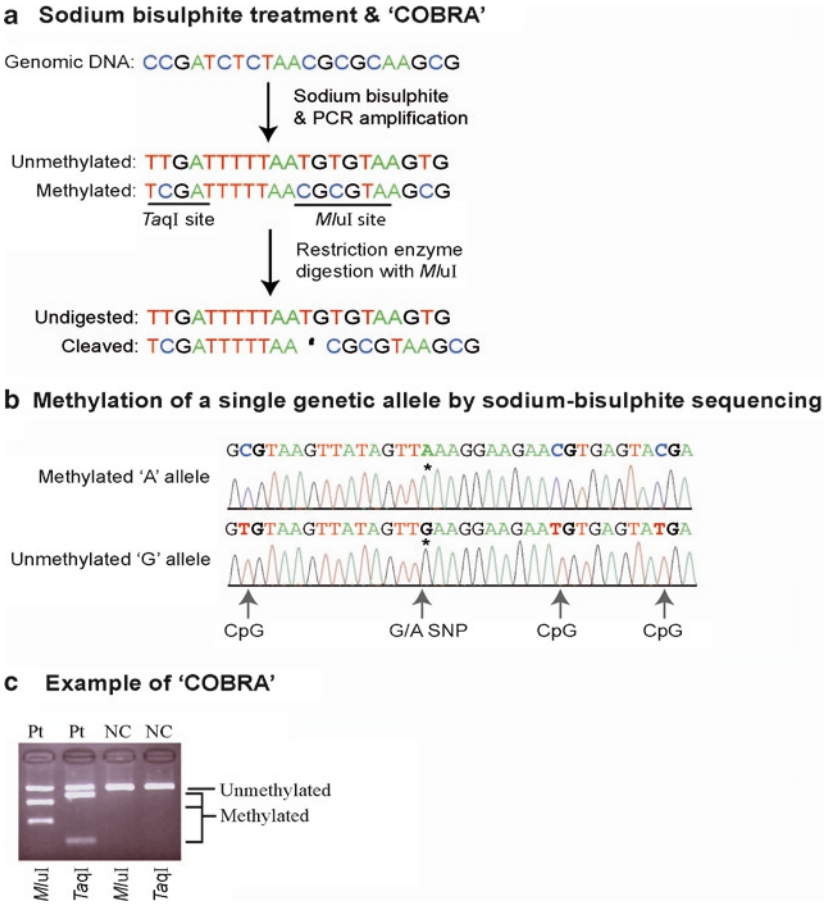


Fig. 2.6 Techniques used to detect and study methylation patterns. (**a**) The process of combined bisulphite and restriction analysis (COBRA) is depicted for a given sequence of genomic DNA, resulting in the digestion of PCR amplified fragments with an enzyme specific to a methylated strand of template DNA. (**b**) Example of a COBRA following gel electrophoresis. A patient with a germline *MLH1* epimutation (Pt) shows a 50:50 banding pattern of methylated (digested) and unmethylated (undigested) alleles, whereas the unmethylated normal control DNA (NC) remains entirely undigested. (**c**) Sequencing of individual alleles of the *MLH1* promoter following sodium bisulphite conversion and cloning of the amplified fragments. The G/A SNP (rs 1800734) within the sequenced fragment and the sites of individual CpG dinucleotides within the original DNA are indicated by *arrows* and *appear in bold type*. In this case, methylation (determined by the presence of CGs) is monoallelic, and associated with the "A" allele. The unmethylated "G" allele is identified beneath by TGs at the CpG sites

2.5.3 *A Newly Described Complication: Inheritance of MLH1 Germline Epimutations*

In cases of germline epimutations, the genetic code of *MLH1* is normal, but the affected allele is transcriptionally inactive and the promoter densely methylated throughout the normal somatic tissues. Thus, in contrast to the predictable

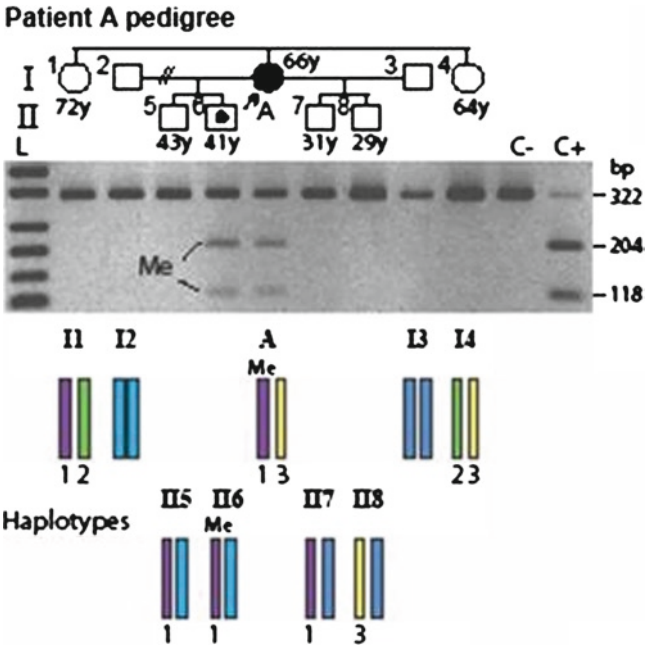


Fig. 2.7 Intergenerational transmission of germline *MLH1* epimutation in Patient A. Patient A's pedigree, with the generations numbered I and II and age of individuals in years (y) is listed. Middle, COBRA showing the presence of methylation (Me) in Patient A and her second son (II6). Below, haplotypes of the family members, with paternal haplotypes shown in *blue*. The epimutation in associated with the *purple* haplotype (allele 1), which is present in five family members, including three sons, but only methylated in Patient A and Son II6, indicating erasure of the epimutation in her two other sons

autosomal dominant pattern of inheritance of sequence mutations, the allele on which a germline epimutation resides can revert back to the normal functional state between generations, due to major epigenetic reprogramming events during the reproductive life-cycle. Germline *MLH1* epimutations tend to arise spontaneously, and since they are reversible during meiosis, are found predominantly in cases without any significant family history [46, 48].

However, a familial case demonstrating non-Mendelian inheritance was described in 2007 in which an affected mother, Patient A, transmitted her *MLH1* epimutation to one of her three sons who each inherited the identical maternal allele [49]. Intriguingly, in the two other sons the epimutation had been cleared and the allele restored to its normal state of activity. Female Patient A presented with multiple primary MSI+ cancers which failed to express the MLH1 protein (Table 2.3). Haplotyping of each family member using informative SNPs within *MLH1* showed that five members of this family had the genetic allele associated with the epimutation (allele 1, Fig. 2.7), including Patient A, her elder sister, and first three sons. Yet only Patient A and her second son had methylation associated with this allele [49]. Furthermore, the epimutation was also reversed in the second son's spermatozoa. In summary, the germline *MLH1* epimutation present in Lynch syndrome Patient A was transmitted to one son, but cleared in his spermatozoa

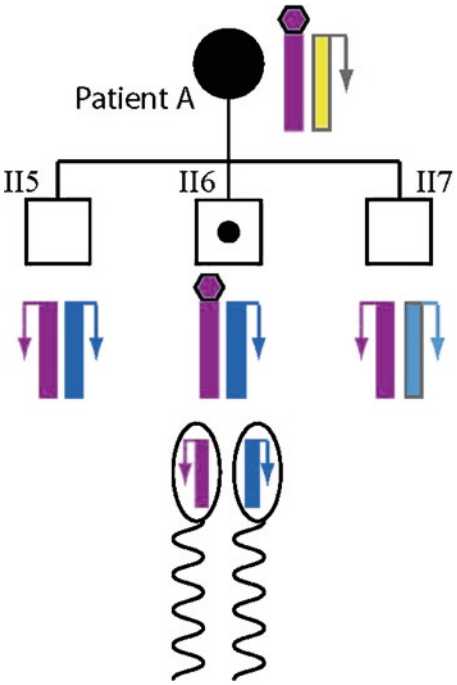


Fig. 2.8 Stochastic inheritance of germline *MLH1* epimutation in Family A. Schematic overview of the transmission and reversal of the germline *MLH1* epimutation in Family A. *Colored bars* indicate different alleles, with *blue shades* representing paternally inherited alleles, the *purple* allele is associated with the epimutation. *Arrows* indicate transcription of the respective alleles. The *purple* allele on which the epimutation was carried was transmitted from Patient A to three of her sons. However, the epimutation itself (*purple* hexagon) was transmitted from Patient A to her second son II6 only, causing transcriptional silencing of the allele in them. The allele was transcribed in the two other sons, II5 and II7, and in the spermatozoa of Son II6, due to clearage of the epimutation

and the allele's expression was reactivated. Two other sons, who also inherited the genetic allele associated with the epimutation, did not inherit the epimutation itself, since the allele had also reverted to normalcy in them (Fig. 2.8).

This family showed that while germline *MLH1* epimutations are usually reversible, they can also be passed from one generation to the next in non-Mendelian fashion. This has confounded traditionally held concepts of disease inheritance and further complicates genetic counseling in Lynch syndrome families with this defect.

2.5.4 Frequency and Screening in the HNPCC Population

Germline epimutations of *MLH1* appear to be rare in Lynch syndrome and are not currently screened for on a routine diagnostic basis [45–50]. Those identified to date have been predominantly sporadic, or have had no significant family history

meeting either the Bethesda or Amsterdam II criteria for Lynch syndrome. No cases of germline *MLH1* epimutations have been identified among probands reaching the Amsterdam I criteria. Yet since this defect confers a similar risk of cancer susceptibility as genetic mutations, and intergenerational transmission has been demonstrated in one family so far, its identification is important in according appropriate clinical management and genetic counseling to carriers and their families. Therefore, it has been proposed that screening should be focussed on selected individuals, irrespective of family history, who have early-onset cancers demonstrating MSI and immunohistochemical loss of the MLH1 protein, and for whom no pathogenic sequence mutation has been identified following genetic screening [48, 50].

Since germline *MLH1* epimutations confer an equivalent risk of developing cancer as genetic sequence mutations of the *MLH1* gene, carriers should receive the same clinical surveillance as Lynch syndrome cases with traditional sequence mutations of this gene. However, the risk of intergenerational transmission appears to be lower than for cases with conventional sequence mutations. Yet, genetic counseling in this scenario is complex. We now know that germline *MLH1* epimutations are heritable through the maternal germline in a stochastic fashion. But while they appear to be erased efficiently in the spermatozoa of affected males [47, 49], this may not necessarily equate to a low risk of inheritance through the paternal germ line if the epimutation can be reimposed on the allele post-fertilization due to genetic interplay. Although the case history of Patient A and her family argue against the involvement of a fully penetrant *cis* defect [49], the influence of a genetic element cannot be ruled out at this stage. Until such a time as the underlying mechanism causing germline epimutations is defined, families should receive cautionary advice that this defect is potentially heritable.

2.5.5 2006: Familial Epimutations of *MSH2* caused by upstream terminal deletions of *EPCAM*

In 2006, an epimutation of the *MSH2* gene was reported in a familial case of Lynch syndrome affecting multiple family members across three generations in whose tumours *MSH2* expression was lost [53]. Like germline *MLH1* epimutations, the *MSH2* epimutation was characterized by promoter methylation of a single allele in the absence of any sequence mutation within the *MSH2* locus. Yet unlike *MLH1* epimutations, with consistent methylation throughout the normal soma, the levels of allelic *MSH2* methylation varied in different somatic tissues, from approximately 3% in peripheral blood to 40% in normal colonic epithelia. Furthermore, the *MSH2* epimutation segregated faithfully with the affected genetic allele, and was transmitted in an autosomal dominant pattern through three generations, implicating an underlying *cis*-acting genetic defect [53]. Linked deletions of the terminal end of the *EPCAM* gene (formerly *TACSTD1*), located immediately upstream of the otherwise intact *MSH2* gene, were subsequently identified in families with "*MSH2* epimutations." These deletions cause continuation of *EPCAM* transcription into

MSH2, ultimately resulting in *MSH2* promoter methylation, predominantly in epithelial tissues where *EPCAM* expression is highest [112].

The finding of germline epimutations of the *MLH1* and *MSH2* genes, in addition to sequence mutations of the five known mismatch repair genes, have revealed that Lynch syndrome is a disease of multiple and complex aetiologies. The recent findings of epigenetic manifestations in the causation of Lynch syndrome may be a sentinel for other diseases previously assumed to be of purely genetic cause.

2.5.6 Effectiveness of Surveillance

Historically, the first large study that showed convincing data on the effectiveness of colonoscopy in Lynch syndrome was that of Järvinen and colleagues [54]. They demonstrated the benefit of colonoscopic screening at 3-year intervals through a controlled clinical trial extending over 15 years. The incidence of CRC was compared in two cohorts of at-risk members of 22 Lynch syndrome families. CRC developed in eight screened subjects (6%), compared with 19 controls (16%; $p=0.014$). The CRC rate was reduced by 62% in those who were screened using colonoscopy. All CRCs in the screened group were local, causing no deaths, compared with nine deaths caused by CRC in the controls. It was concluded that CRC screening at 3-year intervals more than cuts in half the risk of CRC, prevents CRC deaths, and decreases overall mortality by about 65% in Lynch syndrome families.

2.6 Mucosal Architecture

The nature of many biomedical optical technologies has great promise for early diagnosis of colon carcinogenesis in general and specifically to hereditary syndromes such as Lynch syndrome. During development of these technologies, the vast majority of groups focused on the goal of developing the “optical biopsy” – basically to determine the histology of the lesions. This work has continued with data showing that a variety of techniques including light scattering spectroscopy, optical coherence tomography (OCT), or more recently narrow band imaging (NBI). Backman and colleagues [55] developed the ability of using light scattering to probe epithelial architecture. In their initial report they noted that light scattering spectroscopy (LSS) was able to identify dysplastic cells and they followed this up with the demonstration that LSS could detect adenomatous change in the colon [55, 56]. Other conventional techniques had focused on better ways to detect polyps on endoscopy with initial studies on chromoendoscopy being promising [57].

The approach of using light scattering technologies to risk stratify for colonic neoplasia was made possible by Dr. Backman’s development of a powerful suite of light scattering technologies including four-dimensional elastic light scattering

fingerprinting (4D-ELF) and more recently low coherence enhanced light scattering spectroscopy (LEBS) [58, 59]. These technologies allow unprecedented quantitative insights into the nanoscale architecture of the epithelium. It has been previously demonstrated that in the histologically normal mucosa of patients who harbor neoplasia, there are profound proteomic and genomic alterations [60, 61]. Thus, these novel optics approaches allows detection of the nanoscale architectural correlates of the epigenetic/genetic changes of field carcinogenesis.

The promise to risk stratify was first conceived somewhat by serendipity during the analysis of early stages of azoxymethane (AOM)-treated rats. In this model, aberrant crypts foci (ACF) typically require ~5 weeks to develop, adenomas ~20 weeks, and carcinomas ~35–40 weeks. However, at 2 weeks (prior to any morphological/histological markers of neoplasia), profound alterations in the 4D-ELF parameters were noted in the spectral markers [58]. These were further apparent using another light scattering technology, LEBS, which allows depth selectivity in order to target the proliferative compartment of the colonic mucosa where the stem cells are believed to reside. Initial performance characteristics showed a sensitivity of 100% and specificity of 64% for patients with advanced adenomas [59].

Given that this optical approach has been powerful in risk-stratifying for sporadic attention, efforts have been made with hereditary colon cancer. Field carcinogenesis is well established in hereditary syndromes with seminal work by Lynch and colleagues assessing proliferation in patients with Lynch syndrome [62]. The initial studies by Roy and colleagues with the murine model of familial adenomatous polyposis (MIN mouse) showed that both 4D-ELF and LEBS analysis of the histologically normal mucosa distinguished wild-type from MIN mice prior to manifestation of the phenotype with outstanding accuracy [63].

While studies are being conducted on hereditary colon cancer, initial studies have shown that rectal LEBS measurements from the endoscopically normal mucosa were able to distinguish patients with a family history of colon cancer. Moreover, preliminary data on patients with Lynch syndrome indicates that rectal spectral markers for the histologically normal mucosa were markedly abnormal irrespective of the presence of adenomas elsewhere in the colon. Thus, the spectral approach has the potential to identify patients with Lynch syndrome and possibly to assess phenotypic heterogeneity with regards to colon carcinogenesis.

2.7 Value of Knowledge of Mutation Status

A study by Watson et al. [64] showed that cancer risk assessment based on personal and family history of cancer may change significantly with the use of DNA testing for a known mutation in the family. Changes from uncertainty to certainty (that is, to carrier or non-carrier status) accounted for 89% of the risk status changes resulting from testing. Importantly, 60% of family members who had a carrier risk status change were *not* tested themselves but could be reclassified based on a relative's

DNA test result (carrier or non-carrier of the mutation). This is of crucial importance given the fact that such risk changes can significantly affect cancer prevention recommendations, most commonly reducing the financial and personal burden when the at-risk patient is found to be negative for the cancer-causing mutation segregating in his/her family and, conversely, often highly-targeted management opportunities may be possible when positive. While these findings were originally based on families with the hereditary breast–ovarian cancer syndrome and the Lynch syndrome, they are nevertheless of extreme importance in virtually all hereditary cancer syndromes where a culprit mutation has been identified.

2.8 Prophylactic Colectomy

Lynch [65] discussed the role of prophylactic colectomy, basing it upon candidates showing the following features: presence of germline MMR mutation or an obligate germline mutation carrier; lack of compliance with colonoscopy; patient with morbid cancer phobia; and early onset colonic adenoma. Genetic counseling, including detailed discussion of the surgery's rationale and potential sequelae, is essential.

2.9 Prophylactic Hysterectomy and Oophorectomy

Endometrial cancer is now known as a sentinel cancer in the Lynch syndrome [66]. Therein, this problem merits consideration for screening with the option of prophylactic hysterectomy and bilateral salpingo-oophorectomy in women who have completed their families and are consenting for this procedure [67, 68]. They must be fully aware of the limitations of ovarian cancer screening. As in the cases of CRC, genetic counseling and surgical consultation are mandatory.

2.10 Summary

Throughout this brief historical survey of the Lynch syndrome, we have characterized this as if it were a discrete entity. However, careful review of each section of this manuscript reveals significant genotypic and phenotypic heterogeneity. Thus, the lumpers and splitters could have a "field day" in terms of dissecting the syndrome into perhaps multiple configurations such as Lynch syndrome-like, as described by Lindor [40], variations in tumor spectrum due to genotypic heterogeneity wherein *MSH2* shows an increased spectrum of extracolonic cancers in the face of a relative deficit of CRC, while its *MLH1* counterpart shows a relative paucity of extracolonic cancers but an apparent excess of CRCs, and *MSH6* shows an increased frequency of endometrial carcinoma and a relative deficit of CRC and a

generally later age of cancer onset, giving a milder version of the syndrome. Epimutations are discussed at length, given their new historical interest. The evolution of our understanding of the etiology of the Lynch syndrome has spanned a century, beginning with the initial recognition by Warthin that this was a hereditary disease and its subsequent demonstration by Lynch, through the identification of genetic mutations within the various mismatch repair genes, to a new era incorporating epigenetic aberrations in its causation. Where does it all end? Clearly, we project that time will certainly challenge syndromy further, with perhaps several new distinctive characteristics, each of which may represent syndromy unto itself.

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Part II
Basic Sciences and Genetics

Chapter 3
Molecular Genetics of Familial Adenomatous Polyposis

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Abstract With the advances in molecular genetics, the function of the APC gene has been and still is being described. In this chapter, a description of the APC protein, function, its relation to tumorigenesis, Familial Adenomatous Polyposis and other colorectal cancer syndromes will be discussed. Finally animal models which have been proven invaluable in the discovery of the APC protein function, will be described.

Keywords Molecular • Genetics • Familial • Adenomatous • Wnt signalling

3.1 Introduction

The phenotypes of patients with familial polyposis syndromes are remarkably different, in that polyp morphology and associated extra-intestinal features vary greatly between the syndromes. The "freckling" of Peutz–Jeghers syndrome (PJS), for example, has no evident connexion to hamartomatous intestinal polyps and no counterpart in the adenomatous polyposes: familial adenomatous polyposis (FA), attenuated FAP (AFAP), and MYH-associated polyposis (MAP). The connexion between harmartomatous polyps to those tumours of the colon and rectum in the other polyposis syndromes is similarly variable; there are, for example, few reports of Peutz–Jeghers polyps outside PJS. In view of these dissimilarities, it is therefore equally remarkable that all the well-recognised polyposis syndromes predispose to gastrointestinal carcinoma, especially that of the large bowel. It seems self-evident that progression must be through different genetic pathways, for we know that different genes predispose to each polyposis syndrome, yet only in the case of *APC* is there any easy link between the (epi)genetic pathways of polyposis and sporadic tumorigenesis in the colorectum.

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About 25% of the Western population will develop a colorectal tumour by the age of 70, and one or more will progress to malignancy in about 5% of these [1]. However, of the genes that are mutated in the various Mendelian syndromes associated with colorectal cancer, only *APC* and *MLH1* play central roles in the pathogenesis of sporadic tumours. Of these genes, *MLH1* is inactivated by promoter hypermethylation in a minority of several tumour types [2], but *APC* is mutated in the great majority of sporadic colorectal cancers and adenomas, 80% acquiring an *APC* mutation [3], the majority of which occur in the mutation cluster region (MCR) [4]. The molecular biology of the *APC* gene is therefore of evident importance in the study of cancer.

3.2 Some Historical Notes

FAP is the most common polyposis syndrome, with the most florid phenotype. Its tendency to run in families had been noted by Lockhart-Mummery in 1925 [5]. For this reason, once sufficient polymorphisms had been identified in the human genome and the efficacy of linkage analysis had been demonstrated for human diseases, it was natural that the quest to identify the FAP gene should be an early landmark in the study of inherited diseases. At this stage, in the mid-1980s, very few Mendelian disease genes, let alone cancer genes, were known, some important exceptions being p53 and *RB1*. Reasonably good restriction fragment length polymorphisms (RFLP) maps had been constructed [6] and hypervariable minisatellite regions in the genome had been characterised [7], but many polymorphisms were still only identifiable by restriction enzyme digestion and all genotyping required laborious agarose gel electrophoresis or sequencing, followed by radioactive detection. The tracking of alleles through families, as required for linkage analysis, was therefore a task expected to take several years.

Against these inherent problems, the analysis of FAP did present a few advantages over other diseases. There existed in some centres a number of large, well-characterised FAP families with high-penetrance, dominantly inherited disease that were available for study through organisations such as polyposis registries. We also know, in retrospect, that almost all adenomatous polyposis families that were informative for linkage analysis would have been caused by germline mutations in a single gene, and genetic heterogeneity was therefore not a great problem. Furthermore, careful curation had identified individual pedigrees that were large enough on their own to provide a significant linkage signal. In addition, multiple tumours were available for study from colectomy specimens. In the event, despite the dedication of the groups involved, the linkage searches that mapped the *Adenomatous Polyposis Coli (APC) gene* to chromosome 5q21 [8, 9] took several years, despite being aided by the identification of rare patients with germline deletions that inactivated *APC* and that led to other clinical features suggestive of a constitutional cytogenetic abnormality. *APC* was eventually localised to chromosome 5q between bands q21 and q22. Two studies independently obtained these results, the closest polymorphic marker being DP2.5 C11p11.

The concept of a tumour suppressor gene (TSG) and the central role of loss of heterozygosity (LOH) in TSG inactivation had been established by the time that the *APC* gene was localised. Given its autosomal dominant inheritance, the FAP gene was always likely to be a TSG. It was also reasoned correctly – although subsequent findings have revealed a more complex situation for other genes – that FAP adenomas progressed randomly to colorectal carcinoma (CRC) and that FAP adenomas were morphologically identical to sporadic adenomas, sporadic CRCs should harbour *APC* mutations. Since the identity of the *APC* gene was not known at the time that the gene was mapped to 5q21, LOH analysis was the only way of testing this hypothesis. Frequent LOH close to *APC* was found in a series of sporadic CRCs [10], providing good evidence for a role for the gene in the development of sporadic cancers.

The cloning of the *APC* gene was just as difficult and laborious as its localisation, in the absence of a human genome sequence and the need to use radiation hybrids or large, unstable clones such as YACS for physical mapping. Again, the identification of a rare patient with FAP caused by a constitutional deletion of 5q21 was critical to the success of the gene cloning project [11].

3.3 Attenuated FAP

The existence of a milder phenotype than the hundreds or thousands of adenomas in "classical" FAP had been remarked upon before the *APC* gene was cloned. This so-called attenuated phenotype seemed to be highly variable, although some large families were known and inheritance appeared to take Mendelian dominant form. Linkage to chromosome 5q21 could be shown in these families. Thus, the qualitative similarity between the FAP and attenuated FAP phenotype naturally suggested that the latter phenotype may be caused by germline *APC* mutations, as was indeed found to be the case [12]. These AFAP-associated mutations were subsequently shown to be located in specific regions of the *APC* gene (see below).

3.4 Structure of the APC Gene and Protein

APC encodes a large protein, the most common isoform comprising 2,843 amino acids. It comprises 15 principal coding exons (Fig. 3.1). At the protein level, APC shows moderate conservation, being 90% identical to mouse [13], 82% to chicken and 27% to *Drosophila*. Intriguingly, *APC* is absent from yeast and other lower organisms, although it is present in all multicellular organisms. The N-terminus and middle region of APC are the most highly conserved regions. There also exists an APC homologue, APC2, which seems to be expressed exclusively in the brain in humans, although this seems to be the major APC species in *Drosophila.*

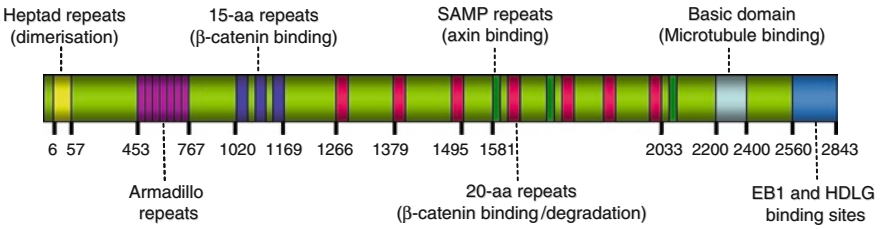

Fig. 3.1 The human APC protein

APC is most strongly transcribed in the central nervous system, and expressed at a lower level in most other tissues. The non-coding 5′ exons 1A and 1B result from alternative transcription initiation sites driven by different promoters [14]. The 1A transcript tends to be the more prevalent, although both isoforms are generally expressed together. The importance of these two isoforms is currently unknown. Several other exons of *APC* are known to be alternatively spliced, the most common being exons 9 and 10A. Again, the importance of these transcripts is unclear, although, unlike the 1A/1B transcripts, different protein isoforms result, the one lacking exon 9, for example, being deficient in one, highly conserved armadillo repeat. The last exon of *APC* is exceptionally large, comprising 6,573 bp and most of the coding sequence (from residue 655 to the 3′ end). The evolutionary reasons (if any) for this unusual structure are not known, but one important consequence may be that most protein-truncating mutations in *APC* are not subjected to nonsense-mediated decay of mRNA, and may therefore be capable of producing stable, truncated proteins.

3.5 APC and Wnt Pathway Regulation

In this section, we briefly describe the role of the normal APC protein.

Intestinal epithelium is regenerated every 3–5 days. Stem cells located at or near the bottom of intestinal crypts produce enterocyte (or colonocyte in the large bowel) and secretory lineages, the latter producing mucus-secreting Goblet cells, antimicrobial Paneth cells and enteroendocrine cells. The enterocyte lineage produces transit amplifying cells and then differentiated cells that perform largely absorptive functions [15]. As enterocytes differentiate, they move up the crypt and, in the small intestine, onto the villus. Finally, cells are shed into the gut lumen [16].

APC is a member of the Wnt signalling pathway (http://www.genome.ad.jp/dbget-bin/show_pathway?hsa04310+5578). Wnt ligands may be derived from stromal or other epithelial cells. After binding to cell-surface receptors of the Frizzled family, a cascade of protein phosphorylation events results in a failure of the APC-axin-GSK3β-CK destruction complex to phosphotag β-catenin for destruction. β-catenin enters the nucleus, dimerises with TCF/LEF1 family transcription factors and promotes expression of target genes such as *MYC*, *CCND1, MMP7* and *AXIN2* (conductin), leading to increased cell division [17, 18].

APC is largely a cytoplasmic protein. Binding to β-catenin is mediated through seven imperfect 20-amino acid repeats motifs, each containing the motif TPXXFSXXXSL, that bind β-catenin [19, 20] after the latter has been phosphorylated by GSK3β [21]. In addition, located between amino acids 1,020 and 1,169 are three 15-amino acid repeats that can also bind β-catenin, albeit more weakly than the 20-amino acid repeats; the function of these repeats remains unclear [20–22].

Three SAMP repeats distal to codon 1580 within the 20-amino-acid repeat region bind AXIN through its N-terminal RGS sequence [23]. The great majority of *APC* mutations found in vivo truncate the protein before codon 1580 and hence remove all SAMP repeats and the ability of APC to form the destruction complex. Like *APC*, AXIN can function as TSG and loss of function through mutation can prevent β-catenin degradation.

3.6 Other Functional Domains of APC

Heptad repeats within the N-terminal oligomerisation domain (first 60 amino acids) allow APC to form homodimers [24]. Relative few data exist on the role and importance of APC dimerisation, although most mutant proteins from colorectal tumours should retain this ability. The N-terminal of APC also contains a highly conserved armadillo repeat domain that can bind several molecules, such as the guanine nucleotide exchange factors 1 and 2 (ASEF1, ASEF2). The armadillo domain of APC is usually retained in APC-mutant tumour cells. However, it has been suggested that truncated APC stimulates ASEF1 more strongly than wild-type APC does, leading to decreased migration of intestinal epithelial cells [25, 26].

Although most APC is in the cytoplasm, it can be found in the nucleus, although the relationship of this localisation to β-catenin-mediated signalling is unclear. The mechanism of APC nuclear transport remains controversial, although there are putative export and important signal sequences at more than one site within APC. It is not known whether nuclear transport is affected by APC mutation, but mutant APC is found in both nucleus and cytoplasm, so any changes are likely to be quantitative.

APC has been shown to regulate cell polarity and migration through control of the actin cytoskeleton. Inactivation of APC leads to decreased cell adhesion. These effects are probably mediated through the C-terminal basic domain of APC (amino acids 2,200–2,400). This region can stimulate polymerisation of tubulin and stabilise the growing ends of microtubules [27, 28]. APC has been described at several microtubule-associated locations, including membrane protrusions and the kinetochore. It may be involved in spindle formation, especially orientation and hence daughter cell polarity and differentiation [29, 30]. Loss of APC causes increased aberrant mitoses and chromosome mis-segregation and rearrangement in vitro. Also close to C-terminus of APC is a domain for binding EB1, a protein that localises to the plus end of microtubules. The final 72 amino acids of the C-terminus of APC contains a PDZ binding motif S/TXV that permits binding of APC to the human homologue of the *Drosophila* discs-large tumour suppressor.

3.7 Germline and Somatic APC Mutations

Disease-associated, germline *APC* mutations almost all truncate the protein. Frameshift changes comprise about 60% of the total, and are about twice as common as nonsense changes which generally involve C>T transitions. Occasional splice-site mutations have been reported. Larger germline deletions involving exons or the whole *APC* gene occur in <10% of cases of FAP, and exonic duplications are even rarer. About 20% of mutations are thought to arise de novo, reflecting the selective disadvantage inherent in FAP. Most germline *APC* mutations occur between codons 168 and 1580, and there are hotspots at codons 1061 and 1309, perhaps because these sites contain short repeat sequences that are prone to spontaneous slippage [31] and/or as a result of genetic drift. The near-absence of mutations distal to codon 1580 is said to reflect the fact that a single remaining SAMP repeat is capable of maintaining the integrity of the β-catenin phosphorylation complex.

APC is somatically mutated in very few tumours other than adenomas and carcinomas of the colorectum. The spectrum of somatic *APC* mutations is similar to that in the germ line, with frameshifts most common, followed by nonsense changes. Interestingly, there is no evidence of a carcinogen mutation signature affecting *APC,* suggesting that most changes are spontaneous events rather than the result of specific dietary factors. There are, however, differences between the locations of the germline and somatic *APC* mutations (Fig. 3.2). While both generally occur before codon 1580, there is only a weak over-representation of codon 1061 and 1309 mutations in the soma. Other sites of short nucleotide repeats tend to be over-represented in the somatic mutation spectrum, including codons 1465 and 1554. Even allowing for some screening bias, there is a strong tendency for somatic mutations to occur between codons 1285 and 1485. The boundaries of this so-called mutation cluster region (MCR) have been subject to some revision and variation over the years, but the existence of the MCR is well established. The reason for its existence is explained below.

Like most tumour suppressors, somatic *APC* mutations often take the form of loss of heterozygosity (LOH). In fact, the frequency of LOH at *APC*, affecting about 30% of CRCs, is lower than for most tumour suppressors, for which LOH is the usual "second hit". *APC* LOH usually occurs by mitotic recombination, a mechanism that involves no change in gene dosage, but leads to duplication of the mutant allele and hence protein inactivation.

3.8 Genotype–Phenotype Associations in FAP

Several genetically heterogeneous Mendelian tumour syndromes exhibit genotype–phenotype associations because mutations at more than one locus can cause phenotypically similar disease. Examples include the tumour spectra in hereditary breast/ovarian cancer (*BRCA1* and *BRCA2*) and neurofibromatosis (*NF1* and *NF2*). Less commonly, different mutations in the same gene cause different phenotypes.

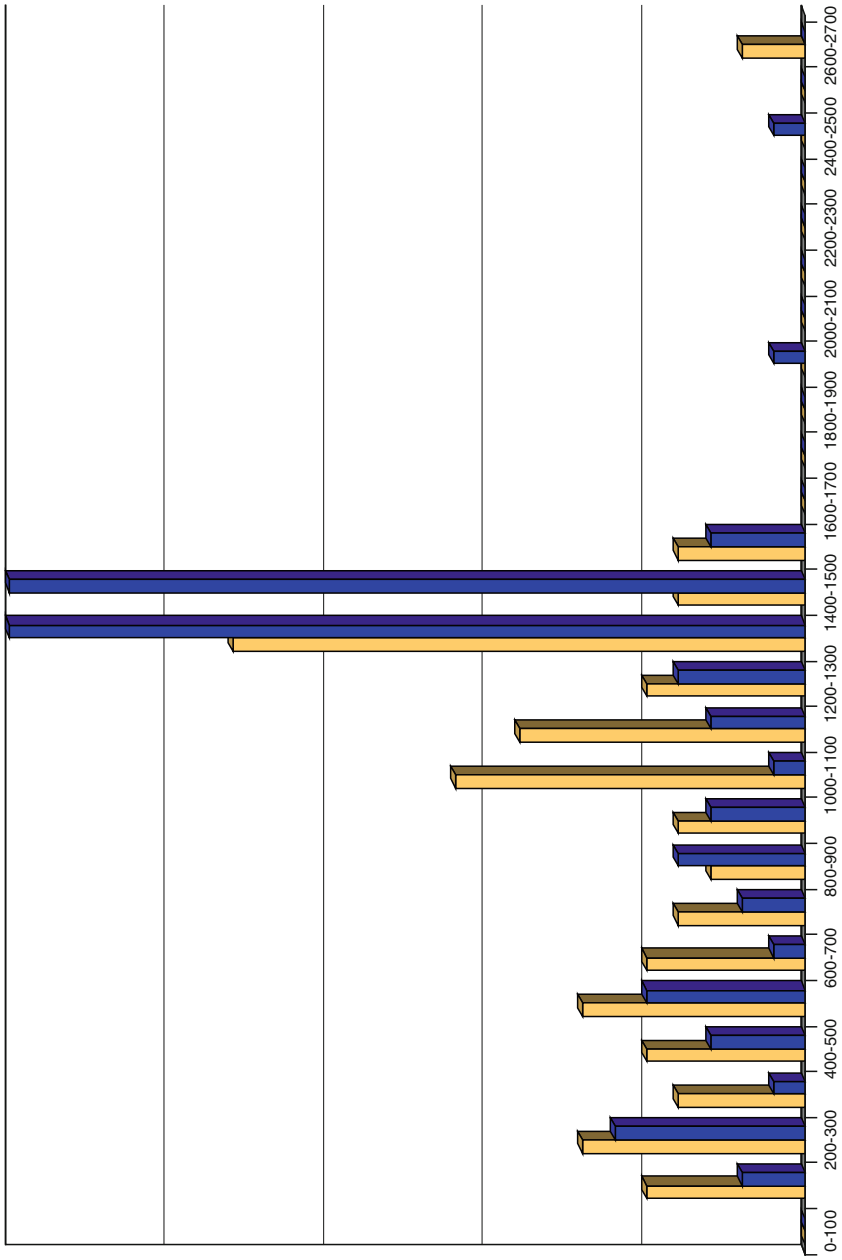

Fig. 3.2 Distribution of germline (*gold*) and somatic (*blue*) *APC* mutations

One example is von Hippel–Lindau (VHL) syndrome, where the location and type (truncating or missense) of germline *VHL* mutations are associated with different tumour spectra. *APC* exhibits a slightly different form of genotype–phenotype association, in that protein-truncating mutations located in different regions of the gene are associated with different number of colonic polyps. There is also evidence that the presence and/or severity of duodenal polyposis, desmoids disease and CHRPE depend on the site of the germline *APC* mutation. However, the number of polyps can also vary even within kindreds with the same germline mutation. It has been proposed that this variation probably results from polymorphisms in other genes that act to modify both the colonic and extra-colonic phenotypes [32].

AFAP-associated mutations generally occur before codon 163 (exons 1–4), within the alternatively spliced part of exon 9 and after codon 1580, distal to the first SAMP repeat. The reasons for the less severe disease are not fully understood. Mutations in exons 1–4 may produce some functional *APC* protein owing to the use of alternative translation initiation sites. In one of these alternative transcripts, exons 1–4 are deleted, in another exons 2–4. Both transcripts use an intra-exonic splice acceptor in exon 5 and the first 16 bases of exon 5 are also lost [33]. Exon 9 mutations may produce a mixture of transcripts, some truncated within exon 9 and others in which exon 9 is spliced out, hence essentially producing a protein of full length. The single SAMP repeat present in proteins encoded by mutations after codon 1580 may, as we have noted, be enough for near-normal Wnt signalling. However, none of these factors really provides a convincing explanation of why disease is attenuated rather than absent, particularly since somatic mutations in AFAP-associated parts of *APC* almost never occur in sporadic tumours. A further complicating factor is that AFAP tumours often acquire "third hits" at *APC,* with loss of the germline mutant allele and mutations at or close to codon 1554 particularly common. Perhaps the principal question is why AFAP cases develop any tumours at all. Presumably, reduced dosage of functional APC protein is important under some circumstances.

Classical FAP is also subject to genotype–phenotype associations. Mutations close to codon 1309 are associated with florid colonic polyposis (typically several thousand adenomas) and early-onset colorectal cancer [34], whereas most other mutations typically produce between 100 and 1,000 adenomas. It has been argued that the association is even more subtle, with mutations between the second and third 20AARs producing severe disease and those between the first and second 20AARs producing very severe disease. This is discussed further below.

Other genotype–phenotype associations have been described in FAP, including more severe upper-gastrointestinal polyposis and desmoid disease in carriers of germline *APC* mutations after codon 1400 [35] and a tendency for CHRPE to occur with germline mutations between exons 9 and 15. However, all genotype–phenotype associations in FAP have only limited clinical utility, because there is considerable variation in disease severity that cannot be explained by the position of the germline mutation. Although environmental exposure and chance cannot be excluded as causes of this variation, the existence of normal inherited genetic polymorphisms (modifier genes) for FAP has been tested [36]. Evidence favours the existence of these genes and shows that differences in disease severity exist at the microadenoma stage, suggesting differences in tumour initiation.

3.9 APC and "Just Right" Wnt Signalling

Although *APC* is a prototypical tumour suppressor gene, it is likely that the somatic mutations found in vivo do not simply inactivate the protein, as is the case for most tumour suppressors. Instead, the first and second "hits" at *APC* are non-random with respect to each other as regards their position within the gene [37]. Specifically, it has been shown for colorectal adenomas and carcinomas that

- "First hits" between the first and second 20AARs are associated with "second hits" by LOH.
- LOH usually occurs by mitotic recombination, resulting in two identical alleles.
- "First hits" before the first 20AAR are associated with "second hits" that leave two 20AARs.
- "First hits" between the second and third 20AARs are associated with "second hits" before the first 20AAR.
- If "three hits" occur, as in AFAP, the eventual combination of mutations generally probably ends up similar to tumours with "two hits".

There is therefore a tendency for human colorectal tumours to acquire mutant APC genes that encode a total of two 20AARs, summed over the two alleles. This is not an absolute requirement, rather a strong tendency. It has been proposed that the associations between allelic *APC* mutations occur in order that there results a level of β-catenin protein, and hence Wnt signalling, that is optimal (or "just right") for tumorigenesis [37–39].

Interestingly, the associations between "first hits" and "second hits" at *APC* are different for extra-colonic tumours in FAP. There is a tendency for duodenal tumours, gastric polyps and desmoids to acquire *APC* mutations that encode a total of four 20AARs. These findings suggest that the optimal level of Wnt signalling for tumorigenesis is different between the colon and the upper gastrointestinal tract and desmoids [40].

The "first hit–second hit" associations at *APC* provide the basis for understanding some of the genotype–phenotype associations in FAP that have been described above. For example, germline mutations between the first and second 20AARs are associated with severe disease and LOH in colonic tumours; mutations between the second and third 20AARs are associated with severe disease and LOH in duodenal tumours and desmoids (Fig. 3.3a). Since LOH occurs relatively frequently and produces a highly selected genotype in such patients, they tend to initiate more tumours than other patients. Similarly, germline mutations between the second and third 20AARs may tend to produce severe, but not very severe, colonic polyposis because their "second hits" can occur anywhere before the first 20AAR, whereas for those with no 20AARs in the germ line, the optimal "second hit" occurs in a small region between the second and third 20AAR (see Fig. 3.3b, c).

Although the seven 20AARs are important for binding of APC to β-catenin, degradation of the latter is dependent on intact SAMP repeats and, as described

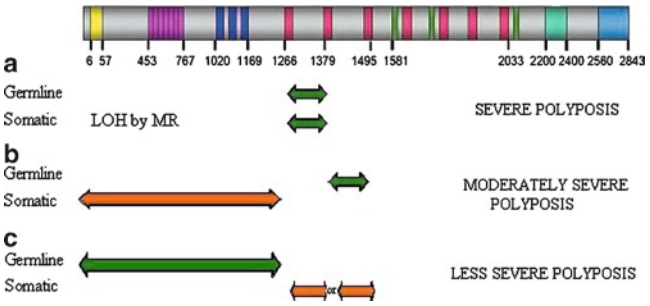

Fig. 3.3 Association between germline and somatic mutations in the APC gene

above, almost all mutant APC proteins found in vivo are truncated before codon 1580 [31]. Failure of β-catenin degradation seems to be at odds with the "first hit–second hit" association. However, most truncated APC proteins are stable, and thus some mutant proteins are potentially capable of binding β-catenin through their remaining 20AAR(s). This binding may be critical for modulating Wnt levels and Schneikert et al. [41] have recently shown that mutant APC can bind β-catenin in vitro and reduce catenin-mediated transcription of a TOPflash reporter, as long as the protein retains one or more 20AARs.

3.10 Missense APC Variants Including I1307K

In addition to the protein-truncating mutations in exons 1–4, exon 9 and distal to codon 1580, a further *APC* variant, I1307K, has been reported to cause an AFAP-like phenotype [42]. I1307K appears to exist mainly in the Ashkenazi Jewish population, with a population prevalence of about 6%. Although a missense change, I1307K is located in a critical region of *APC*, suggesting some functional effect. However, the generally favoured disease mechanism is that I1307K creates an A_8 tract that is hypermutable in somatic cells owing to replication-induced errors and gain/loss of an adenine, resulting in a frameshift change. Recent reports suggest that the degree of hypermutation is small, with the overall excess risk of colorectal cancer only about 1.5-fold, if not less [43]. Nevertheless, several individuals or families with I1307K and an AFAP-like phenotype have been reported. It is notable, however, that not all of the polyps from these I1307K carriers acquire a slippage of the A_8 tract [44]. These observations may result from a combination of ascertainment bias, variation between individuals in the tendency for the A_8 tract to slip, and co-inheritance of a separate predisposition to colorectal tumours (although an *APC* mutation in linkage disequilibrium with I1307K is unlikely). Upper-gastrointestinal disease and desmoids appear to be uncommon or rare in *APC* I1307K carriers.

The missense variant E1317Q is present in many populations with an allele frequency of about 1%. Initially promising data suggested that this variant may be associated with colorectal adenoma and carcinoma risk, but subsequent reports

have failed to confirm the initial data. It has become clear that the association between E1317Q and adenomas came from a chance association with *MYH* mutations. In fact, it is arguable that *MYH*-associated polyposis (MAP) would not have been discovered without E1317Q, since the initial investigations of the first MAP family were actually focussed on working out whether E1317Q was functional.

APC polymorphisms have been proposed on several occasions as low-penetrance colorectal tumour susceptibility alleles. Most intragenic polymorphisms result in synonymous products as regards the APC protein, although a small number of non-synonymous proteins exists. D1822V is a common *APC* variant that has been analysed in several association studies. Although some suggestive effects on bowel cancer risk have been detected, larger studies have found very little evidence of any effect of either D1822V or any other polymorphism within or around *APC*.

3.11 APC Promoter Hypermethylation

In addition to point mutations and small and large deletion, tumour suppressors can also be inactivated by epi-genetic silencing of their promoters. This is achieved by modification of the methylation patterns in these regions. As mentioned earlier, the most common mechanisms of APC somatic inactivation in FAP (and in sporadic) colorectal tumours involve protein truncating mutations and LOH. However, a considerable fraction of colorectal tumours do not have detectable second hits suggesting that an additional mechanism is involved in APC inactivation. Previous studies have shown of the two APC promoters, 1A is the most commonly active in colonic mucosa [45]. This promoter has been found to be hypermethylated in colorectal adenomas and carcinomas [46]. Tumours with methylated APC do not seem to express the APC protein or express the protein at low levels [47–49] On the basis of this observation, it has been proposed that methylation is a third mechanism of somatic inactivation of APC. Unlike in HNPCC where methylation has been observed in the soma and the germline, no inheritable APC germline epigenetic mutations have been reported in FAP.

3.12 APC Mutations in MYH-Associated Polyposis

MYH is a glycosylase that is part of the base excision repair machinery of the cell. Oxidative damage can cause the mutant base 8-oxoguanine to be erroneously incorporated into DNA in place of guanine. MYH removes adenine residues that have been incorporated opposite 8-oxogunaine owing to a tendency for these bases to mispair. If MYH is deficient, following replication, thymidine can be incorporated opposite the adenine. Consequently, germline *MYH* mutations, which lead to absent or severely deficient glycosylase activity, cause an excess of G:C>T:A nonsense mutations in *APC,* leading to a polyposis phenotype [50]. MAP tumours develop along a specific genetic pathway characterised by G:C>T:A somatic hypermutation

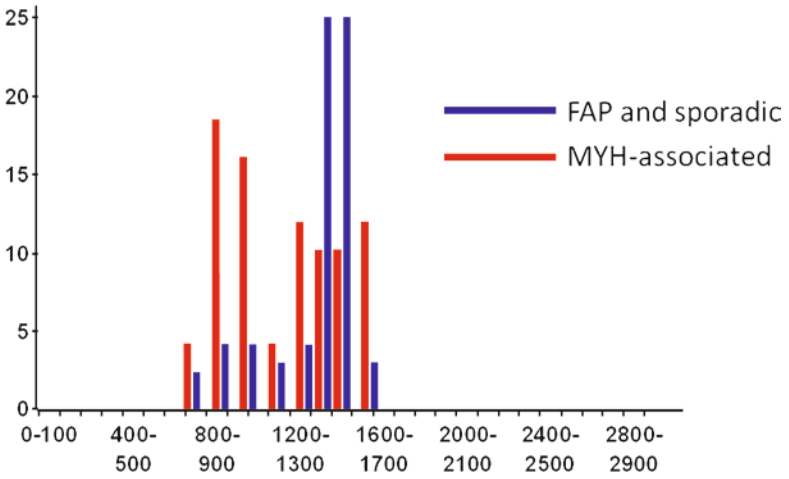

Fig. 3.4 Locations of somatic *APC* mutations in MAP tumours and FAP/sporadic colorectal tumours

and therefore somatic bi-allelic mutations at *APC* are the norm, with a low frequency of LOH. Nevertheless, MAP tumours still follow the "just right" model. This causes the distribution of somatic *APC* mutations in MAP to differ from those in FAP and sporadic colorectal tumours, with a much less obvious MCR (Fig. 3.4).

3.13 Mouse Models of Intestinal Polyposis

The Multiple Intestinal Neoplasia (*Min*) mouse, which develops significant numbers of adenomas mostly in the small intestine, was identified following random mutagenesis with ethylnitrosourea [51]. A truncating mutation at codon 850 of *Apc* carried as heterozygote mutation – homozygosity for mutant *Apc* results in embryonic lethality – was responsible for a phenotype that resembles human FAP with the exception of tumour concentration in the small bowel and lack of progression before death: *Min* mice develop relatively few colonic adenomas that only occasionally progress to invasive adenocarcinoma [51, 52] while human FAP patients have predominantly colonic adenomas, which routinely progress [52–54]. No metastasis has been reported [55]. The Apc^{Min} mutation initiates mitotic defects in histologically normal crypt cells of the murine small intestine, with misoriented spindles, misaligned chromosomes, and tetraploid cells observed [53]. Adenoma formation usually follows loss of the wild-type APC copy as a consequence of whole chromosome 18 deletion.

Distinct human kindreds with identical mutant *APC* alleles are often diverse with respect to colonic polyp burden, a feature thought to be due to the environment, particularly the diet. However, genetic background has been shown to influence

strongly the tumour burden in the *Min* mouse. For example, $Apc^{Min/+}$ on the C57BL/6J background develop an average of 29 tumours at death, while the progeny of these mice crossed to AKR animals average as few as six. Further breeding experiments involving backcrossing mice has shown that genes exist that alter tumour multiplicity, distribution and size [54, 56, 57]. Substantial efforts have been made to isolate such "modifiers" as a prelude to identifying the equivalent genetic elements in humans. To date, five modifiers of *Min* (Mom) have been identified: *Mom1–3* and *Mom6* and *Mom7*. The *Mom1* locus was mapped to distal chromosome 4 [56], shown to act in a semi-dominant fashion [58] and to be non-cell autonomous, namely to act outside the tumour lineage [58]. The locus was further refined to a region that included the *phospholipase A2* (*Pla2g2a)* gene [59], but subsequent work suggested that this secretory phospholipase only partially defined a complex locus, which possibly contained two or more two other tightly linked, additive modifiers [60] that differentially influenced tumorigenesis in the subregions of the gut. The second, undefined region (*Mom6*) would appear to be the only influence on tumorigenesis in the medial region of the small intestine [60]. No statistical association between polymorphisms in human *PLA2G2A* and the prevalence of human intestinal tumours has yet been reported [61] nor have genes syntenic to the *Mom6* region been studied in human FAP.

A second modifier of the Min phenotype, *Mom2*, was serendipitously detected when a breeding pair produced offspring with a bimodal distribution of polyp burden [62]. A spontaneously arising mutation mapped to distal chromosome 18 conferred a dominant, resistant phenotype with reduced polyp multiplicity with stronger effects on gut tumour multiplicity than *Mom1*. Fine mapping excluded members of the SMAD gene family, *Madh2*, *Madh4* and *Madh7,* plus *Tcf4* and *DCC,* and resolved the *Mom2* interval to 1 Mb. Sequencing of genes within this interval revealed 4-base pair duplication in the coding sequence of ATPase component of *Atp5a1* leading to loss of function [63]. Loss of the wild-type copy through whole chromosome 18 deletion led to cell death and protection against adenoma formation. As this gene is important to cell survival, it is unlikely that an equivalent loss of function mutation will be found in human population studies, although hypomorphic alleles of this gene, or other family members, may be important in the aetiology of human polyposis and cancer.

A third modifier of Min, *Mom3*, was linked to *Apc*, most likely centromeric to *Apc* on chromosome 18 [64]. Interestingly, a difference in frequency of wild-type allele loss was demonstrated between the recombinant lines that showed clearly defined high and low levels of tumour multiplicity, suggesting that the modifier might be a structural element influencing whole chromosome deletion. Similarly, *Mom7* may also have a role in mediating LOH owing to its position close to the centromere of chromosome 18 and thereby modulating net tumour growth or initiation through influencing loss of wild-type *Apc* [65].

Following on from the *Min* mouse, and with improvements in the technologies necessary to generate mouse models, various mutations in the *Apc* gene have been engineered which, to a limited extent, reflect mutations found in FAP kindreds and sporadic colorectal cancers (Table 3.1, Fig. 3.5). Common to all models is the requirement for loss of the wild-type *Apc* allele so that tumour development can take place.

Table 3.1 Mouse models with germline *Apc* mutations

GEM	Mutation	Polyp burden[a]	Histology/pathology	Comments	Reference
Apc^{Min}	Protein truncated at codon 850	~30 to >100	Polypoid, papillary, and sessile adenomas. Cystic crypts, no ACFs. Mammary adenocathanoma	Canonical FAP model	[51]
Apc$^{D716/+}$	Protein truncated at codon 716	~300	Polypoid, papillary, and sessile adenomas. No ACFs	Normal villous epithelium covering each polyp	[55, 68]
Apc$^{1638N/+}$	Protein truncated at codon 1638	<10	Polypoid and hyperplastic polyps Moderate to highly differentiated adenocarcinoma, infiltration into mucosa and submucosa. Gastric lesions and a single liver metastasis. Desmoids, cutaneous cysts and ACFs	Expression of truncated protein not detected	[55, 70, 93, 94]
Apc$^{1638T/+}$	Protein truncated at codon 1638	–	No intestinal tumours. Smaller body size, absence of preputial glands, nipple-associated cysts	Expression of 182 kDa protein	[94]
Apc$^{1309/+}$	Frameshift at codon 1309	34	Polyps throughout duodenum to colon	More colonic polyps than Apc$^{Min/+}$	[71]
Apc^{D14}	Frameshift at codon 580	~65	Colonic polyps, ACFs. Tubular adenomas and invasive carcinomas after 12 months	More colonic polyps than Apc$^{Min/+}$	[67]

Apc^{580D}	Frameshift at codon 580	~6	Adenomas, mostly near anus	Induction may account for tumour localisation	[95]
Apc^{D580}	Frameshift at codon 580 and truncation at codon 605	~120	Intestinal phenotype similar to Apc^{D14} abnormalities in the skin, thymus and tooth	Similar extra-colonic features to FAP patients (tooth defects)	[96]
Apc$^{D474/+}$	Duplication of exons 7, 8, 9 and 10. Frameshift at codon 474	~30–100	Sessile polyps. Mammary adenocathanoma (18% at 3–5 months). Hyper-proliferation of intestinal glands		[72]
ApcneoR *ApcneoF*	Inserted into intron 13, in reverse or forward orientation	<1 at 15 months		Hypomorphic alleles reduce Apc by 80 and 90% respectively	[97]

GEM genetically engineered mouse
[a]Each mouse

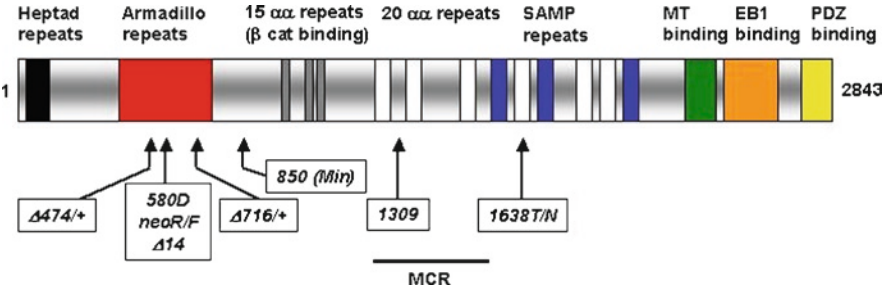

Fig. 3.5 Position of Mouse *Apc* mutations with respect to APC protein domains. Mouse mutants are indicated by a *black arrow* and genotype. Human mutation cluster region (MCR) is from approximately 1,250–1,550 aa [72, 73]. The 15AAR (aa) repeats are in *grey*, the 20 AAR are in *white* and SAMP repeats are in *blue*

Although the histology of intestinal tumours is similar, and largely independent of mutation position, the onset, multiplicity (when account is taken of genetic background) and tumour location is dependent on where the protein is mutated [55]. For example, $Apc^{Min/+}$, $Apc^{D716/+}$ and $Apc^{1638N/+}$ mice produce polyps that are histologically indistinguishable from each other, but there are substantial differences in tumour numbers when each mutation is carried on the same genetic background [66]. Care should be taken when assessing the influence of the mutation on Apc protein levels and localised gene effects. For example, the targeting cassette is not excised from the $Apc^{D716/+}$, which may influence gene expression up- and downstream of Neo [67]. The normal villous epithelium surrounding individual polyps in this model was considered to indicate failures in tissue building that were also responsible for the increased, variable and adenoma numbers [68]. Nuclear β-catenin staining was predominantly nuclear in the $Apc^{D716/+}$ mouse [69]. The $Apc^{1638N/+}$ mutants have a reduced polyp burden allowing increased life span and, consequently, tumour progression [70]. The $Apc^{1309/+}$ mouse does show an increased colonic polyp burden compared to other models and FAP patients with a hotspot mutation at codon 1309 generally show a 10-year earlier onset of disease [71].

Apc deficiency is associated with increased crypt size, cell proliferation and apoptosis and, in addition, cells in Apc-deficient crypts show reduced crypt-to-villus migration and differentiation [74]. Compound *Apc* mouse models have shown that the proto-oncogene *Myc* has a crucial role in the early stages of Apc-driven intestinal neoplasia. Inducible, concurrent loss of both *Apc* and *Myc* demonstrated that loss of *Myc* could abolish the early stage crypt phenotypes linked to Apc deficiency. This study also identified a subset of Wnt target genes that require *Myc* for regulation, independent of the increase in β-catenin levels [75]. Studies using an inducible Apc-deficient mouse revealed that the downstream target of Wnt signalling, *Ccnd1*, was not upregulated immediately following *Apc* loss. A compound $Ccnd1^{-/-}$, inducible Apc deficient model confirmed that Cyclin D1 did not contribute to the early crypt phenotype associated with Apc loss [76]. However, Cyclin D1 deficiency did reduce adenoma burden [76, 77].

The short lifespan of the mouse limits tumour progression even when adenoma numbers are reduced to prevent premature death. Compound mutant models (Apc plus another germline mutation in a gene associated with human cancer) have been used to enhance tumour progression, to provide information on the adenoma to carcinoma transition and to study the influence of genomic instability of which there are two types, chromosomal and microsatellite. The former results in losses and/or gains of chromosomal regions, while the latter is characteristic of mismatch repair (MMR) deficiency and slippage and di- and mono-nucleotide repeat regions. Both types of instability have been shown to enhance intestinal neoplasia in *Apc* mouse models. While MMR deficiency modelled by knocking out various MMR genes, *Mlh1*, *Msh2*, *Msh3*, *Msh6* and *Pms2* [78–81] does not result in significant numbers of intestinal neoplasias, dramatic increases in small intestinal tumour burden are often found when combined with Apc deficiency [82–84]. In $Mlh1^{-/-}$ $Apc^{1638N/+}$ compound mutants, tumour multiplicity is increased, and tumour grade higher with 30% of tumours classified as early invasive cancers or adenocarcinomas [85]. Rather than LOH, adenomas from these compound models show somatic mutations, including frameshifts and base substitutions that account for 70–80% of wild-type Apc protein loss [82–84].

The limited invasive potential of tumours in *Apc* mouse models, has focussed attention on genes known to be important in the adenoma–carcinoma transition such as K-ras, mutated in 40–50% of all human colorectal cancers [86] but not in polyps *Apc* mouse adenomas [87, 88]. Apc-deficient mice (Apc^{580D}) also carrying an oncogenic K-ras allele had 17% of tumours develop into adenocarcinoma [86]. Loss of EphB receptors occurs in transition to carcinoma in humans. By combining a dominant negative *EphB2* transgene with Apc^{Min}, compound heterozygous mice showed more than a tenfold increase in macroscopic colon tumours, which all displayed mucosal invasion [89]. Deficiency for the Smad family of proteins has also been implicated in malignant progression in *Apc* mouse models. Compound *Apc* mutant mice with disruptions in *Smad2* ($Smad2^{+/-}$ $Apc^{580D/+}$), and *Smad4* ($Smad4^{+/-}$ $Apc^{\Delta 716}$ and $Smad4^{+/E6sad}$ $Apc^{+/1638N}$) all showed increased tumour invasiveness compared to the relevant *Apc* model [90–92]. Both *Smad4* and *Apc* map to chromosome 18, and the phenotype was more severe if the affected alleles were in *cis,* where LOH would result in loss of both wild-type genes, rather than *trans*. In the *Smad4* compound model, over 50% of tumours in *cis*-compound mice showed submucosal invasion compared with 10–15% in the equivalent *Smad2* compound heterozygotes [91]. Despite the increased invasiveness, no *Apc* compound models consistently display metastasis. Modelling metastasis is a significant challenge, and it remains to be seen whether suitable compound mutant mice can be developed.

3.14 Concluding Remarks

The functions of the *APC* gene and protein have slowly been unravelled since the discovery that it was the gene mutated in FAP. Mutations almost all inactivate the protein, and thus activate Wnt signalling. However, the mutations rarely remove all

function. This results in genotype–phenotype associations, the mutation cluster region, the low frequency of LOH at *APC* and the association between the first and second hits in FAP and sporadic colorectal tumours. The amount of Wnt signalling that is best at promoting tumour growth is unclear. The importance for tumorigenesis of inactivating other functions of APC, such as its role in chromosome segregation, is unclear. Moreover, we have few clues as to why *APC* mutations are also exclusively associated with large- and small-bowel tumours, while tumours of many other sites acquire activated Wnt signalling through β-catenin mutations. The link between defective base excision repair, colorectal adenomas and *APC* is similarly perplexing. It is tempting to suggest that the study of *APC* molecular genetics is close to its limit, although this gene continues to produce new insights with general relevance to cancer biology. It is fair, however, to state that advances in APC biology are more likely to come from studies with a functional element, and we look forward to the resolution in the next few years of issues such as the role of APC in chromosomal instability and in stem cell maintenance and differentiation.

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Chapter 4
DNA Mismatch Repair

C. Richard Boland

Abstract The genome is subject to multiple forms of stress and damage that can lead to alterations in the integrity of DNA. The cell nucleus possesses several complex, integrated enzyme systems that identify altered DNA and repair it or, in the case of overwhelming damage, trigger cell death, which prevents the passage of a mutation to the next generation of cells. These systems are essential for the faithful replication of the genome, and germline defects in these systems give rise to familial cancer syndromes, each with a unique spectrum of neoplasia.

Keywords DNA mismatch repair • Mutation • Microsatellite instability • MSH2 • MLH1 • MSH6 • PMS2 • Lynch syndrome • Exonuclease

4.1 DNA Damage and Repair

The genome is subject to multiple forms of stress and damage that can lead to alterations in the integrity of DNA. The cell nucleus possesses several complex, integrated enzyme systems that identify altered DNA and repair it or, in the case of overwhelming damage, trigger cell death, which prevents the passage of a mutation to the next generation of cells. These systems are essential for the faithful replication of the genome, and germline defects in these systems give rise to familial cancer syndromes, each with a unique spectrum of neoplasia.

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4.1.1 Types of DNA Damage and Specific Repair Mechanisms

Depending upon the environmental stresses, there can be multiple different forms of DNA damage, and several biochemical systems have been identified with specialized repair activities.

4.1.1.1 Nucleotide Excision Repair

Nucleotide excision repair (NER) was the first recognized system of DNA repair, and was discovered to be essential for recovery from DNA damage induced by ultraviolet (UV) light. NER requires a family of enzymes for complete activity. One of the characteristic types of DNA damage in response to UV irradiation is the generation of dipyrimidine dimers, which covalently link adjacent pyrimidine residues and prevent transcription and replication. NER must break the DNA chain to initiate repair, and larger segments of DNA may be excised as this reaction proceeds.

In humans, homozygous inactivating mutations in one of these genes leads to a complete loss of NER activity (i.e., it behaves in a recessive fashion) and causes the disease xeroderma pigmentosa, in which there is an extraordinary sensitivity to sunlight. As there are multiple different enzymes involved in NER, individual families may have germline mutations in different genes. Gene discovery was facilitated by finding complementing and non-complementing cell types in different affected individuals. Nuclear extracts from one affected individual would restore NER activity in extracts from individuals affected at different enzyme loci, whereas extracts from patients with defects in the same gene would not. The concept of complementary and non-complementary defects is important to the understanding of germline mutations in complex biochemical systems such as those involved in DNA repair.

4.1.1.2 Base Excision Repair

The base excision repair (BER) system includes several DNA glycolases that remove damaged or altered nucleotide bases, and restore the authentic sequence as dictated by the unaltered template strand. This is particularly important for the repair of apurinic or apyrimidinic sites that develop spontaneously under physiological conditions on a continuous basis. BER is initiated by DNA glycosylases, which remove individual free bases, and there are numerous enzymes in this system, each of which has a unique specificity.

In humans, homozygous inactivating mutations in one of the BER enzymes, the *MutY homolog* (or *MYH*), lead to *MYH*-associated polyposis of the colon [1]. In this instance, the germline mutations are in the *MYH* genes, but the target mutations (which are the proximate mediators of carcinogenesis) are in standard tumor suppressor genes, such as *APC* and *p53*. Interestingly, the basis of this disorder was predicted from an analysis of the mutational signatures in the colorectal neoplasms in this disease, and the recognition that the loss of BER activity would result in this specific spectrum of mutations [1].

4.1.1.3 DNA Mismatch Repair

DNA mismatch repair (MMR) is a system that recognizes and repairs classes of DNA damage that differ, at least in part, from damage repaired by NER or BER. Therefore, defects in each of these systems results in a distinct "signature" pattern of mutations in the tumor DNA. DNA MMR is present from *Escherichia coli*, through all higher organisms. MMR has become more complex through evolution, but the fundamental genes involved (the MutS and MutL families) have retained a high degree of sequence and functional homology.

DNA MMR is responsible for repairing replicative errors in DNA that occur during S phase, and DNA heteroduplex mismatches that occur during homologous recombination, which can occur during meiosis in eukaryotes. The principal types of S phase errors recognized by DNA MMR are simple base mismatches, and insertion/deletion mismatches that are accidentally created by DNA polymerase. This repair system can also recognize and repair DNA altered by the spontaneous deamination of 5-methylcytosine, which creates a thymine and results in a mutagenic G-T mispair.

4.1.2 Evolutionary Considerations in the DNA MMR System

The DNA MMR system is more complex in eukaryotes than prokaryotes, but the system is highly conserved from yeast to humans. The complexity found in eukaryotes is due, in part, to the duplication of the *MutS* and *MutL* genes followed by the generation of genetic diversity over time, and selection for advantageous biological properties. The principal mismatch recognition protein in *E. coli* is the Mut S protein, which forms a homodimer that recognizes specific types of DNA mismatches. Yeast, such as *Saccharomyces cerevisiae*, have a wider spectrum of mismatch repair capability, due to the formation of protein heteroduplexes between different MutS homologs, which permits recognition of a wider range of DNA mismatches, resulting in a high degree of genomic integrity [2].

4.1.3 Mismatch Recognition Proteins: The Mut-S Family

4.1.3.1 Mismatch Recognition in Prokaryotes

The *MutS* homodimers found in *E. coli* have been replaced by heterodimers between homologs of the parent proteins in higher organisms; there are no functioning MutS homodimers in eukaryotes. In humans, the obligatory member of the partnership is the human MutS homolog 2 (MSH2) protein, which dimerizes with either human MutS homolog 6 (MSH6) creating the MutSα heterodimer, or human MutS homolog 3 (MSH3), giving rise to the MutSβ heterodimer (Fig. 4.1).

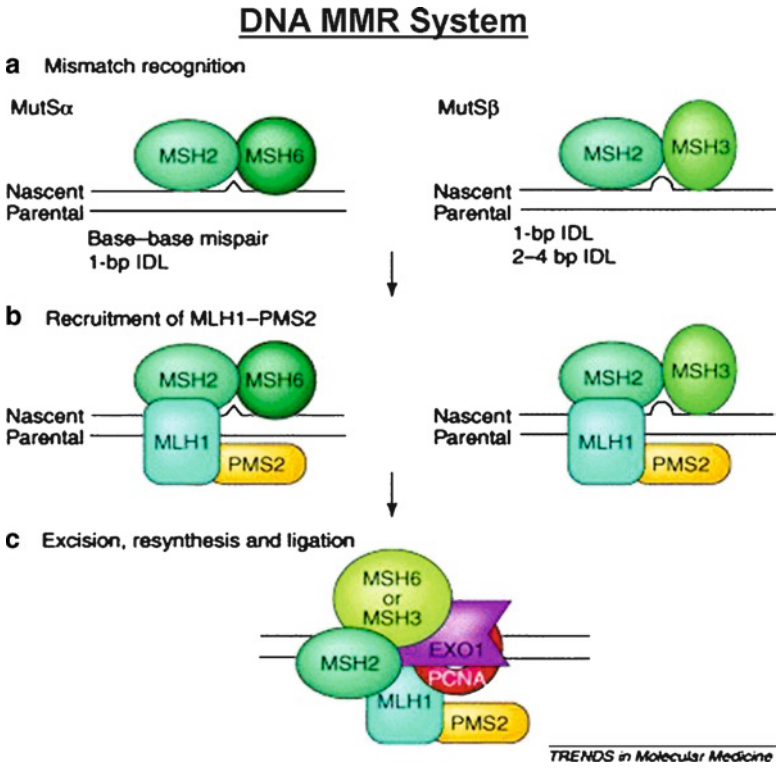

Fig. 4.1 DNA mismatch repair proteins. DNA MMR activity can be divided into three components: (**a**) mismatch recognition by Mut S homologs, (**b**) recruitment and matchmaking with Mut L homologs, and (**c**) excision, resynthesis and ligation of the double helix. Taken from Wei et al. [60]. (**a**) On the *left*, the MutSα dimer of MSH2 and MSH6 recognizes and binds to a single base-pair mismatch. For example, should the DNA polymerase insert a T on the nascent strand across from a G on the template strand, this would alter the physical characteristics of the double helix, which would be recognized by MutSα. On the *right*, the MutSβ heterodimer has specificity for small insertion-deletion loops, or IDLs, in which the loop-out ranges from 1-4 base pairs. Larger loop-outs are dealt with by other proteins. (**b**) Once the mispair or loop-out is bound by the MutS dimers, a protein complex involving a MutLα heterodimer of MLH1 and PMS2 is recruited to the DNA-MutS complex. (**c**) The MutS proteins have identified the site of the mismatch, and together with the MutL proteins, the complex interacts with PCNA of the replication complex on actively replicating DNA, and brings the exonuclease Exo1 to the complex, which permits excision of all of the newly synthesized nucleotides on the nascent strand, resynthesis by the native DNA polymerase complex, and finally, ligation of the gaps between Okazaki fragments

DNA mismatch recognition occurs via heterodimers in higher organisms.

MutSα has a particular affinity for recognizing single base-pair mismatches such as G-T mispairing. In fact, the initial name for the MSH6 protein was the "G-T binding protein," or GTBP [3]. The MutSβ heterodimer has high affinity for recognizing insertion/deletion "loop out" errors that can occur at repetitive sequences during DNA synthesis. For example, if there is a DNA sequence consisting of a repetitive

string of adenines (a poly-A tract, such as …AAAAAAAAAAAAAAAA…), while the DNA polymerase is attempting to replicate this, "slippage" can occur, and some of the template can be skipped, creating a loop-out of the template (which would result in a deletion mutation in the newly synthesized poly-T strand). Alternatively, the polymerase could add too many thymines on the nascent strand and create a "loop out" on the poly-T strand, resulting in an insertion mutation. MutSβ provides additional recognition specificity for errors involving larger loop-outs, including (but not confined to) CA-GT dinucleotide repeats (such as …CACACACACACACACA… etc.), which are particularly common in the human genome. Although either insertion or deletion mutations may occur at repetitive sequences during replication, deletion mutations are much more common when DNA MMR is defective, and this serves as a "mutational signature" for defective DNA MMR.

There is some degree of overlap in DNA mismatch recognition between MutSα and MutSβ, and based upon inference from the clinical spectrum in Lynch Syndrome, MutSα appears to have a broader spectrum of mismatch recognition activity than MutSβ.

4.1.3.2 Stabilization of MutS Proteins

MutS monomers are unstable until a dimer is formed, but MSH2 is the "major" protein, and is required for a stable complex. If there is no MSH2, then one can find no MutSα or MutSβ in the cell [4]. If there is an isolated absence of MSH6 protein, MSH2 is stabilized by interacting with MSH3; conversely, in the isolated absence of MSH3, MSH2 is stabilized by heterodimerizing with more MSH6. Therefore, MSH2 is essential for DNA MMR activity, but MSH6 or MSH3 are relatively dispensable, as one can replace the other. It requires defects in both of these "minor" MutS homologs to inactivate the DNA MMR system [4], although germline MSH6 mutations can be pathogenic, and cause Lynch Syndrome-MSH6 type.

4.1.3.3 Recognition of Mismatched DNA by MutS Heterodimers

The recognition of damaged DNA is an energy-requiring reaction. Free MutSα and MutSβ are bound by ADP, diffuse through the nucleus in an "open" configuration, and interact with DNA in the nucleus (Figs. 4.2 and 4.3). Mismatches and loop-out errors in newly synthesized DNA physically deform the double helix, but also create excess flexibility in DNA, which is recognized by the MutSα and MutSβ heterodimers. An interaction between a DNA mismatch and open-configuration MutS complexes triggers an energy-requiring reaction in which ATP is exchanged for the ADP on the protein complex. This triggers a change in conformation, converting the MutS complex to a "sliding clamp" that encircles the nascent DNA strand [5–7]. This serves to "tag" the location of the DNA mismatch, and targets the area to be repaired.

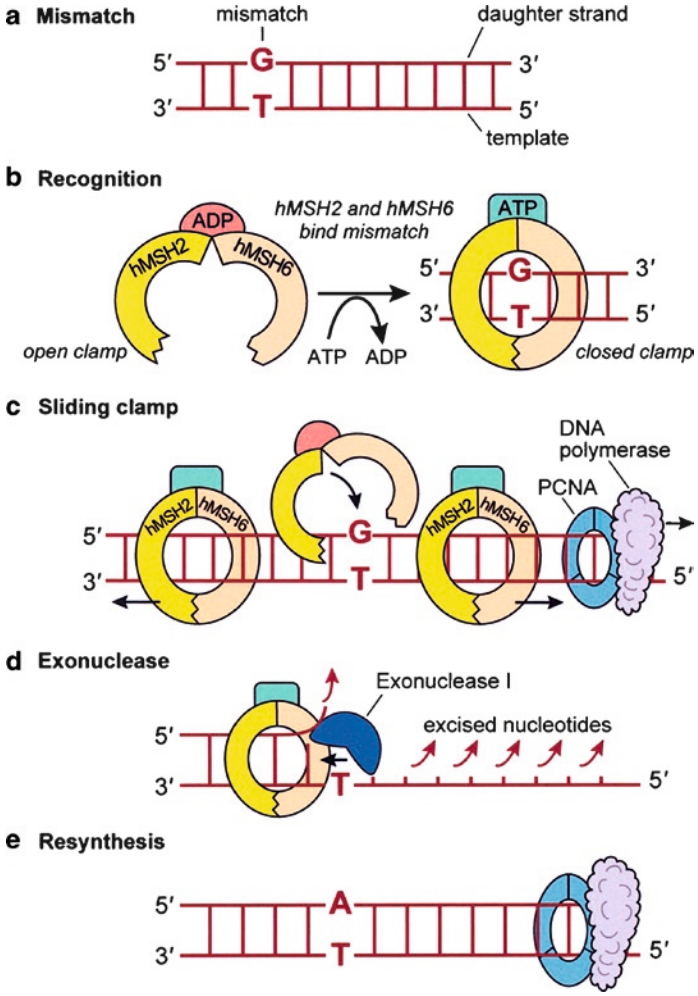

Fig. 4.2 Sliding clamps on newly synthesized DNA. (**a**) The DNA polymerase has created a G–T mismatch by incorporating a G on the daughter strand across from a T on the template. (**b**) The heterodimer of MSH2 and MSH6, bound by ADP and in an open configuration, monitors newly synthesized DNA strand for mispairs. Upon encountering the G–T mispair, an exchange of ATP for ADP occurs and MutSα switches to a closed, sliding clamp that can diffuse quickly along the DNA. (**c**) The sliding clamps can migrate in either direction from the mispair, and as this occurs, additional MutSα clamps may be recruited to the mismatch. The MutSα moving in the 5′ to 3′ direction will eventually encounter the PCNA-DNA polymerase complex, and according to one hypothesis, displace the enzymes involved in DNA synthesis. (**d**) Exonuclease I (ExoI) excises the newly synthesized daughter strand back to the site of the mismatch, eventually removing the potentially mutagenic G. (**e**) The error is corrected by resynthesis using the native DNA polymerase enzyme complex. Originally adapted from Fishel [61], taken from the review by Jascur and Boland [2]

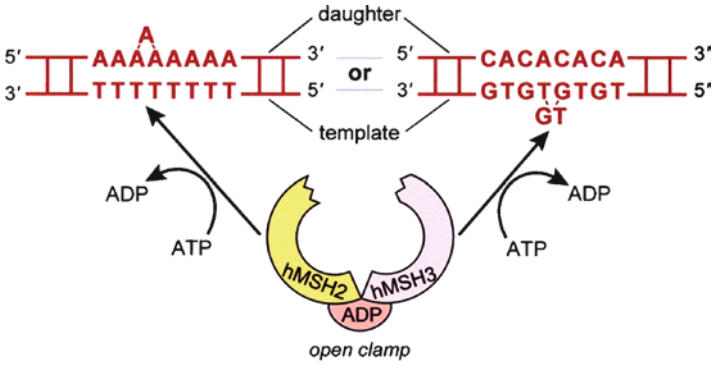

Fig. 4.3 Repair of insertion-deletion loops (IDLs) by DNA MMR. IDL lesions are caused by "slippage" during DNA replication, are recognized by MSH2+MSH3, and corrected. Mononucleotide repeats such as A_n (which is T_n on the complementary strand) on the *left*, or dinucleotide repeats (such as $\{CA:GT\}_n$) on the *right* are recognized by MutSβ, triggering ATP–ADP exchange, long-patch excision, and resynthesis. For purposes of illustration, the slippage has created a short "loop out" on the nascent strand for the A_n sequence, which would lead to an insertion frameshift mutation after replication, and on the template strand for the $(CA)_n$ repeat, which would lead to a deletion mutation. This particular error is best recognized by the MSH2+MSH6 heterodimer. The heterodimer of MSH2+MSH3 has greater affinity for larger insertion/deletion loops (not shown here) that commonly occur during DNA replication at microsatellite sequences. Originally adapted from Fishel [61], taken from the review by Jascur and Boland [2]

4.1.3.4 Other MutS Homologs

There are several other members of the MutS homolog family (yeast genes MSH1 are involved in mitochondrial DNA repair; MSH4 and MSH5 are involved in meiotic recombination; MSH7), but they do not appear to play an important role in standard DNA MMR in humans.

4.1.4 Matchmaker Proteins: The Mut-L Family

The next step in DNA MMR is less clear, but it requires members of the MutL family of proteins. The presence of MutSα or MutSβ as sliding clamps on DNA triggers the recruitment of MutL complexes to the site of mismatched DNA, which then recruit the other proteins required to excise the error and permit resynthesis of the DNA. As is the case for the MutS proteins, MutL proteins function as homodimers in *E. coli*, but there are several MutL homologs that heterodimerize in humans, and no MutL homodimers function in eukaryotic cells. The most important is the *human MutL homolog 1* (*MLH1*), which is the "major" MutL protein in DNA MMR (similar to MSH2 for the MutS system). MLH1 heterodimerizes with other members of the MutL family, including the

human post-meiotic segregation 2 gene (called *PMS2*, although its sequence is actually most homologous to the yeast *pms1* gene), the *human post-meiotic segregation 1* gene (called *PMS1*), and the *human MutL homolog 3* (called *MLH3*).

The heterodimer of MLH1 and PMS2 is called MutLα, and is the most important of the DNA MMR matchmakers. The functions and significance of the other heterodimers (MLH1 + PMS1 = MutLβ, and MLH1 + MLH3 = MutLγ) are unknown at this time. It is reasonable to speculate that these other heterodimers facilitate additional functions in defense of the integrity of DNA or participate in the recruitment of specific proteins required for repairing single base-pair mismatches or larger insertion-deletion loop-outs [8].

Again, similar to the situation with MutS proteins, MLH1 is required to stabilize PMS2, PMS1, or MLH3. The converse is not the case, and the individual absence of PMS2, PMS1, or MLH3 is not sufficient to abrogate DNA MMR activity [4].

4.1.4.1 Molecular Matchmakers

MutLα associates with MutSα or MutSβ at the sites of DNA mismatches, and recruits additional members of the DNA MMR family to the DNA strand containing the error. Thus, they appear to serve as molecular matchmakers (illustrated in Fig. 4.4). The key proteins recruited by the MutL complexes are: (a) the proliferating cell nuclear antigen (PCNA), which forms a homo-trimer that encircles DNA like a clamp and facilitates DNA polymerase activity; and (b) exonuclease-1 (EXO1), which is required to excise the newly synthesized strand that contains the error. There are several conceptual models addressing how this occurs [8, 9], but the details remain obscure.

4.1.4.2 Excision of the Error from the Mismatch, and Strand Discrimination

The DNA MMR system excises DNA from the newly synthesized strand beginning at the site of the error (directed by MutS recognition complexes), and correctly distinguishes the nascent strand (containing the error) from the template strand (encoding the correct sequence). In *E. coli* and other gram negative bacteria, this is achieved through the MutH protein, which binds to newly synthesized DNA strand until it becomes methylated at the end of DNA replication. The mechanism used in higher organisms is not yet clear, but the MMR system may recognize the newly synthesized strand by locating the gaps between Okazaki fragments which are a consequence of discontinuous DNA synthesis on the lagging strand at replication forks.

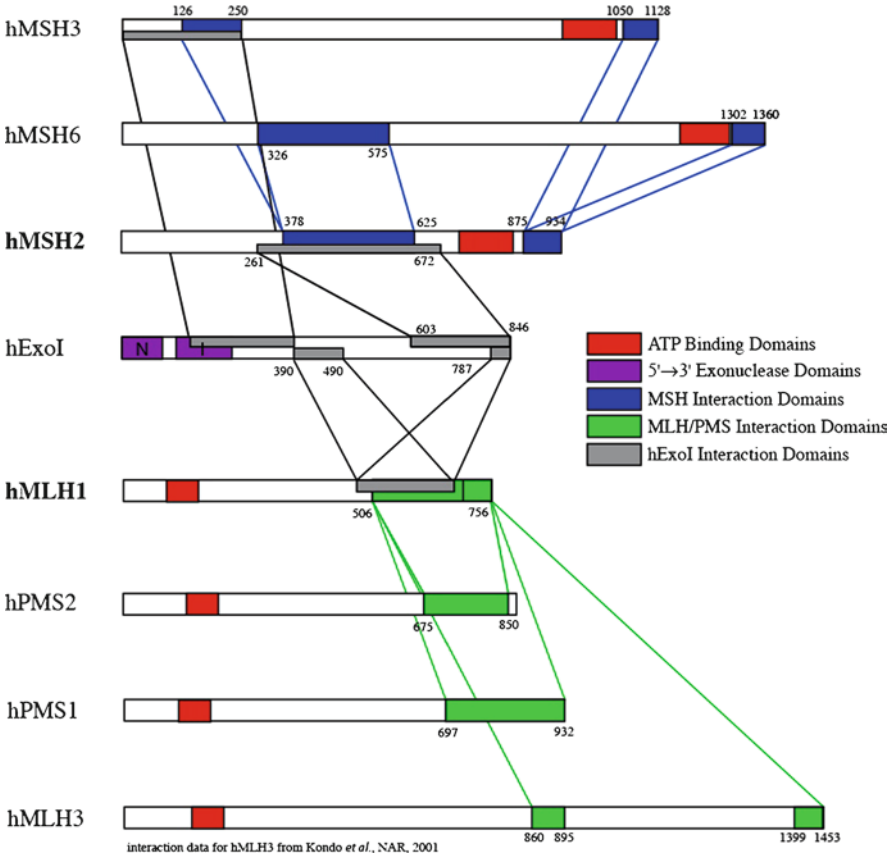

Fig. 4.4 Interactive domains among the DNA MMR proteins. The interaction regions among the human MutS homologs (MSH) are shown in the *upper three bars*. The interaction regions between the human MutL homologs (MLH/PMS) are shown in the *bottom four bars*. The interaction regions between the human excision exonuclease hEXOI and the human MSH and MLH/PMS proteins are shown in the *middle*. Biochemical studies have shown that the MLH/PMS heterodimer will only form an active repair complex with the ATP-bound MSH heterodimer sliding clamps. The binding of ATP by the MLH/PMS heterodimer then appears to stabilize an interaction between hEXOI with the active MSH-MLH/PMS complex to perform the DNA excision step of MMR. The ATP binding domains of both MSH (Walker-like) and MLH/PMS (GHKL-like) proteins are indicated through the interconnecting *vertical-slanted lines*. Mutations in the ATP binding domains and interaction regions are candidates for loss-of-function, and Lynch Syndrome genes. Taken from Boland and Fishel [62]

4.2 Microsatellite Instability

4.2.1 Microsatellites

The human genome contains a very large number of repetitive sequences. It is estimated that there are $>10^5$ dinucleotide repeats (e.g., $[CA]_n$), and a smaller number of mononucleotide repeats (e.g., $[A]_n$), where n equals the number of repeated

elements in the tract [10]. These sequences are highly prone to deletion or insertion mutations during new DNA synthesis, and the faithful replication of these sequences is maintained, in large part, by DNA MMR activity.

4.2.2 Loss of DNA MMR Causes MSI

The absence or deficiency of DNA MMR activity permits the excess accumulation of certain types of mutations by a factor of ~100-fold. One can appreciate this aberration by extracting the DNA from a tumor specimen that has defective DNA MMR activity, amplifying microsatellite sequences by the polymerase chain reaction (PCR), and comparing this with normal DNA from that individual. The DNA from the cancer contains deletions in microsatellite sequence, which can serve as a surrogate for the absence of DNA MMR activity (Fig. 4.5). This phenotype is called microsatellite instability (MSI), and is a hallmark of the DNA from Lynch Syndrome tumors [11, 12]. If one were to acutely inactivate

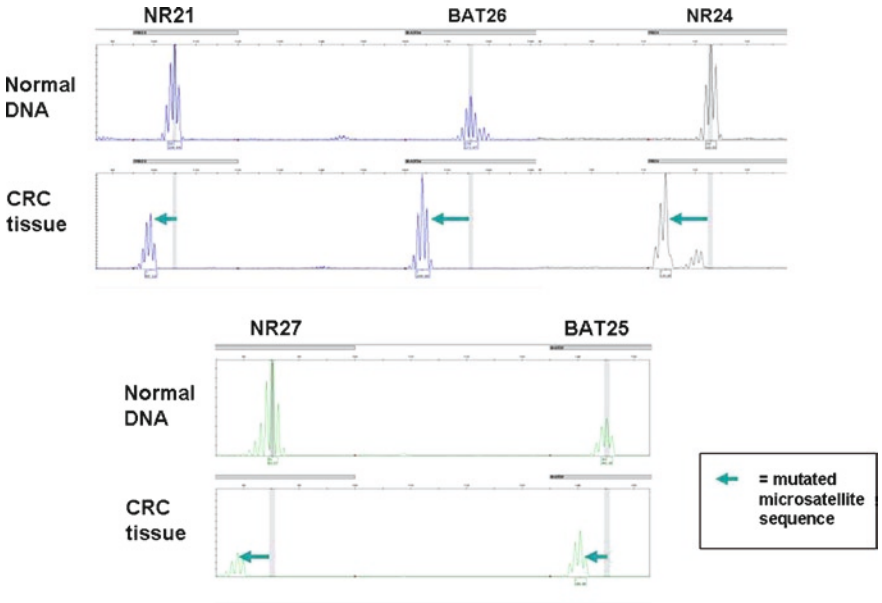

Fig. 4.5 Microsatellite instability. DNA from normal colonic tissue and CRC were extracted from a paraffin-embedded specimen, and PCR was used to amplify five loci in a single pentaplex reaction. The target loci are all mononucleotide repeats: NR21, BAT26, NR24, NR27, and BAT25. In each instance, the PRC product, or amplicon, from the normal DNA eluted (separated by HPCL using a sequencing column) in the expected range, as shown in the *upper lanes*. Below each is the matched amplicon from the tumor DNA, and each has experienced a deletion mutation, and elutes earlier, as indicated by the *arrow*. In this case, all five loci are mutated, or unstable, and this tumor is MSI-H

the DNA MMR system, MSI would not be identified. It takes time for the mutated microsatellite sequences to undergo clonal expansion, which usually occurs passively, as most of these sequences are not thought to be of functional significance for the survival of a cell. In most instances, they undergo clonal expansion as "passengers" in cells that have gained a growth advantage. However, some microsatellite sequences occur in coding regions, and mutations in target genes actually mediate neoplastic behavior, as discussed below (see: Molecular Targets of MSI in Cancer).

4.2.3 The Discovery of MSI in Colorectal Cancer

The DNA repair systems were discovered in bacteria and yeast, which facilitated the characterization of the biochemistry of each system, and eventually, the cloning of the genes involved. The micro-organisms with DNA repair defects had a highly mutagenic phenotype. As a consequence of this defect, the organisms are more tolerant of genotoxic attacks, and this provides mechanisms to generate genetic diversity, and survive DNA damage [13].

However, it was not immediately obvious to those working on hereditary colon cancer that defects in the human homologues of these genes would necessarily lead to an increased predisposition to cancer. In fact, the linkage of defective DNA MMR to familial colorectal cancer (CRC) occurred, to some degree, by accident.

In 1993, two laboratories had independently discovered MSI as a phenotype that could be found in about 12–15% of CRCs, and recognized that these tumors had unique clinical characteristics; however they were initially unaware of the linkage to hereditary CRC [10, 14].

4.2.4 MSI Is Linked to Hereditary CRC

At about the same time, an international consortium of investigators was performing genome-wide linkage analysis on families with Lynch Syndrome, and linked the occurrence of early-onset CRC in a large kindred to a locus on chromosome 2p [15]. They then used microsatellite amplification to look for loss of the wild-type allele as the second hit at this locus, but instead of finding loss of heterozygosity (LOH), they encountered MSI, which provided a link to the possible pathophysiology of carcinogenesis in this setting [16]. In a remarkably short period of time, the 2p22-21 locus was found to encode the MSH2 protein [17, 18], and shortly thereafter, the MLH1 gene was found to be responsible for the defect in another group of colorectal cancer families in which disease was linked to chromosome 3p21-23 [19, 20]. Moreover, two other DNA MMR genes responsible for Lynch Syndrome were found by sequence homology, and these were the PMS1 (2q31-33) and PMS2 (7p22) genes [21], the latter of which has been linked to Lynch Syndrome families.

Not long thereafter, the MSH6 gene (located on 2p, about 1 megabase from MSH2) was linked to some hereditary cancer families as well, especially those with a relatively strong tendency to endometrial cancer as well as CRC [22, 23]. PMS2, and MSH6 mutant tumors sometimes displayed a lower level of instability than those with MSH2 or MLH1 mutations.

It was subsequently appreciated that most CRCs with MSI were not related to Lynch Syndrome, but were caused by epigenetic silencing of the MLH1 gene [24, 25].

4.2.5 DNA MMR Genes and Lynch Syndrome

There has been a movement toward the use of the term Lynch Syndrome for the hereditary cancer syndrome that is caused by a germline mutation in a DNA MMR gene [26]. It is currently appreciated that germline mutations in four of the DNA MMR genes – MSH2, MLH1, MSH6, and PMS2 (Table 4.1) – cause Lynch Syndrome. The phenotype and clinical features vary considerably, depending in part on which gene is mutated [27].

4.2.5.1 Major and Minor DNA MMR Genes

Germline mutations in the two "major" DNA MMR genes, MSH2 and MLH1, are responsible for most of the families with Lynch Syndrome [28, 29]. The "minor" DNA MMR genes, MSH6 and PMS2, are responsible for fewer familial clusters of cancer, because they are partially expendable in the DNA MMR process, and can be partially replaced by the other MutS and MutL homologs. Thus, the clinical phenotypes associated with germline mutations in the "minor genes" are attenuated, although the reasons for the existence of variable phenotypes remain obscure. In the case of Lynch Syndrome-MSH6 type, the cancer phenotype is attenuated in time, and

Table 4.1 DNA MMR and Lynch syndrome

DNA MMR genes in which germline mutations may cause Lynch syndrome
MSH2
MSH6
MLH1
PMS2 [33, 34]
DNA MMR-associated genes not definitively linked to Lynch syndrome families
MSH3 [63]
PMS1 [64]
MLH3 (controversial, but certainly uncommon [65–67])
Exo1 (probably not [68, 69])
PCNA

the cancers occur later in life. However, by the eighth decade, the cumulative cancer incidence is approximately the same as occurs in Lynch Syndrome-MSH2 type or -MLH1 type [23, 30]. Because of the limited impact of MSH6 loss on DNA MMR, these tumors may show low-level MSI (MSI-L), or even be microsatellite stable (MSS) [30]. This was predicted from observations that cultured cells defective at the MSH6 locus had lower levels of MSI at mononucleotide repeats, and almost none at dinucleotide repeats [31].

Relatively few families have been reported with germline mutations in the PMS2 gene, but this is due, in part, to the presence of multiple PMS2 pseudogenes in the genome, which obscures diagnostic strategies on a technical basis [32]. However, if one performs immunohistochemistry on CRC specimens, approximately 1.5% of all tumors show isolated loss of PMS2 protein expression, a finding consistent with germline mutations in that gene [33, 34]. This observation implies that germline mutations in PMS2 are as common as those in MSH2 or MLH1, but are simply less well recognized clinically [34]. A solution to this problem will require additional data.

Germline mutations in DNA MMR genes typically occur in one allele, and Lynch Syndrome is inherited as a classic autosomal dominant predisposition to multiple, early-onset cancers. The normal tissues of a patient with Lynch Syndrome do not show MSI, as one allele is sufficient to produce a normal DNA MMR phenotype. At the tissue level, however, the disease takes on a recessive quality, as inactivation of the second, wild-type allele is required for loss of DNA MMR and the evolution of MSI. This can occur through allelic loss [35], an epigenetic silencing of the wild-type allele [36], or presumably, a second mutation.

Rarely, an individual inherits a mutant allele from both parents, and has homozygous or compound heterozygous mutations at both alleles of a DNA MMR gene. This has been reported for all four of the DNA MMR genes [37–40]. These individuals have no DNA MMR activity, and have MSI in normal tissues. The phenotype is extremely severe, with childhood cancers (including leukemias, lymphomas, and very early onset colon tumors). Interestingly, the children may have café-au-lait spots resembling those seen in neurofibromatosis.

4.3 Molecular Targets of MSI in Cancer

As mentioned above, MSI *per se* does not cause cancer. However, it has been argued that MMR deficiency affects pathways involved in programmed cell death, and may directly affect cell behavior. The best documented mediators of the neoplastic phenotype are the genes or DNA sequences that happen to encode microsatellite sequences in critical regions, and are involved in the regulation of cell growth and other behaviors. Table 4.2 provides a list of target genes that are frequently mutated in CRCs with MSI. The list includes genes critical for the regulation of growth such as TGF-βRII (which harbors an A_{10} sequence), IGF2R (a G_8 sequence), BAX (a G_8 sequence), TCF4 (an A_9 sequence), and curiously, several genes involved in DNA repair including MSH2, MSH3, MSH6, PMS2, and RAD50 [41].

Table 4.2 Target genes encoding repetitive sequences sensitive to defective DNA MMR

Coding repeat and genes affected
Poly-adenine tracts
A_{10}
AIM2
CASPASE 5
MBD4
SEC63 (and A_9)
TGFβRII
A_9
BLM
CHK1
GRB-14
MLH3 (and A_8) [70]
RAD50
RHAMM
RIZ (and A_8)
SEC63 (and A_{10})
TCF-4
WISP3
A_8
ACVRII (two such sequences, only one frequently mutated [71])
APAF-1
BCL-10
hG4-1
MLH3 (and A_9)
MSH3 (and A_7) [70]
PMS2
RIZ (and A_9)
A_7
MSH2 [70]
MSH3 (and A_8)
MSH6 (and C_8, T_7) [70]
A_6
PTEN (has two A_6 sequences)
AXIN-2 (has two A_6 sequences, plus G_7 and C_6)
Poly-thymidine tracts
T_{10}
OGT
T_9
KIAA0971
NADH-UOB
T_7
FAS
MSH6 (and C_8, A_7)

(continued)

Table 4.2 (continued)

Coding repeat and genes affected
Poly-cytidine tracts
C_9
SLC23A1
C_8
MSH6 (and A_7, T_7)
C_6
AXIN-2 (and two A_6 and G_7)
Poly-guanine tracts
G_8
BAX
IGF2R
G_7
AXIN-2 (also two A_6 sequences, and C_6)
CDX2

The following genes have mononucleotide runs of at least six repetitive nucleotides, and the list includes a number of DNA repair genes (such as MSH2, MSH6, MSH3, MLH3, MBD4, BLM, CHK1, RAD50, APAF), several that are critical to apoptosis (caspase-5, BCL-10, hG4-1, RIZ, PTEN, FAS, BAX), and several critical tumor suppressor genes (TGFβRII, IGF2R, AXIN-2) or transcription factors (TCF-4, CDX2) [41]. Deletions mutations have been reported in most of these sequences in MSI-H CRCs, which create frameshifts and premature stop codons, inactivating the gene products. These are the actual functional targets of MSI that lead to neoplastic behavior. Adapted from refs. [41, 70, 71]

It has been suggested that the loss of DNA MMR activity leads to a "mutator phenotype," in which the cell progressively accumulates inactivating mutations in these genes, thus driving progressive, multistage carcinogenesis in such tumors [42]. This makes the MSI phenotype a specific pathway in cancer development, a concept that was appreciated from its initial recognition [14]. Although the individual genes inactivated by mutation differ from those found in the more common pathways (such as chromosomal instability [43] or CIN [44]), the same signaling pathways are typically altered in MSI+ AND MSI− neoplasms. Occasionally, the same gene may be inactivated in CRCs from each of these three pathways, but by different mechanisms. Specifically, the *APC* gene, which is a key regulator of WNT signaling, can be inactivated by allelic loss [45], by mutation at a repetitive sequence [46], or by methylation [47]. Many tumors have more than one mutational mechanism affecting *APC*, as biallelic inactivation is required for this tumor suppressor gene.

4.4 Defective MSI in Non-Lynch Syndrome CRC

About 15% of all CRCs have MSI, but at most one quarter of these (or 3–4% of the total) result from Lynch Syndrome. The majority have DNA MMR deficiency due to silencing of *MLH1* expression by CpG island methylation [24, 25, 48]. These CRCs have the MSI+ phenotype, do not express either MLH1 or PMS2 protein, and have

mutations in most of the same target genes as are seen in Lynch Syndrome tumors. One notable exception is a characteristic mutation in the BRAF gene (V600E), which is essentially never seen in Lynch Syndrome CRCs, but occurs in a relatively large proportion of non-Lynch Syndrome CRCs with silencing of *MLH1* [49–51]. Thus, most CRCs with MSI are not attributable to Lynch Syndrome, and this represents the single biggest clinical challenge when using MSI testing to screen patients for Lynch Syndrome. CRCs with acquired *MLH1* silencing tend to occur in older patients (facilitating the distinction from Lynch Syndrome), about 90% of the tumors are in the proximal colon (whereas a less pronounced bias occurs in Lynch Syndrome), and the survival is somewhat better than MLH1-expressing (microsatellite stable) CRCs [10, 14].

4.5 Hereditary CRC Without Defective DNA MMR ("Familial Colorectal Cancer, Type X")

Not all hereditary clusters of CRC are caused by germline mutations in a DNA MMR gene. Among all families that meet the Amsterdam Criteria for hereditary CRC, about 60% have an identifiable mutation in either MSH2, MLH1, or MSH6 [52, 53]. The other 40% are familial clusters of CRC that do not have increased incidences of the other cancers seen in Lynch Syndrome. Furthermore, the penetrance for cancer is lower, and the age of onset is somewhat later. These families have been labeled "familial CRC – type X," and the genetic basis of this disease is not known. It is easily distinguished by the consistent absence of MSI in the CRCs. Many of the so-called "familial CRC" families may be chance clusters of CRC, and the epithet may not be beneficial.

4.6 Pathological Downregulation of DNA MMR Activity

Chronic mucosal inflammation is associated with an increased risk of cancer in the colon and elsewhere in the gastrointestinal tract. Of interest, the DNA from biopsies of patients with chronic ulcerative colitis show a very high frequency of low-level MSI (called MSI-L), whereas this is not seen in acute self-limited colitis, in normal mucosa from control patients, or in patients with Lynch Syndrome [54–56]. These patients do not have germline mutations in DNA MMR genes. This observation raised the possibility that the DNA MMR system may be downregulated in the setting of chronic inflammation. Two models of oxidative stress that reproduce at least part of the milieu of chronic inflammation have been developed, and both lead to a down-regulation or "relaxation" of the DNA MMR system [57, 58]. Oxidative stress increases the mutational load to DNA, and relaxation of DNA MMR adds to the risk of developing deleterious mutations in target genes. The CRCs that develop in the setting of ulcerative colitis do not frequently have the MSI-H signature [55, 59], indicating that the impact of relaxed DNA MMR activity is distinct from complete inactivation of the system.

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Chapter 5
Basic Sciences and Genetics: Hamartomatous Polyposis

James R. Howe and Daniel Calva

Abstract It can be challenging to distinguish between the various hamartomatous polyposis syndromes based upon the histology of polyps, as not all patients present with pathognomonic signs of one of these specific conditions. The discovery of several causative genes for the hamartomatous polyposis syndromes has added a new dimension to the classification of these patients, allowing for demarcation on a molecular basis. Clinicians can then be aware of other anomalies to look for, and can perform presymptomatic diagnosis of at-risk individuals. This chapter will cover the history of the discovery of the predisposing genes, the types of mutations found, the function of these genes, and genotype–phenotype correlations identified for the major hamartomatous polyposis syndromes.

Keywords Juvenile polyposis • Cowden syndrome • Peutz–Jeghers syndrome • Hamartomatous polyposis • Genetics • Gastric cancer • Colorectal cancer • SMAD4 • Bone morphogenetic protein receptor (BMPR1A) • Autosomal dominant

5.1 Juvenile Polyposis

During the early part of the twentieth century, there was confusion about the classification of juvenile polyps, which were thought to be adenomatous or congenital [1]. Roth and Helwig described 158 patients with juvenile polyps in 1963, found in 99 children and 59 adults [2]. Fourteen percent were diagnosed with

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multiple polyps, and therefore might have had Juvenile Polyposis (JP), but screening of the colon at this time was not systematic, and therefore most cases were likely patients with solitary juvenile polyps. McColl et al. defined the term Juvenile Polyposis Coli in 1964 to differentiate solitary juvenile or retention polyps from those with multiple juvenile polyps [3]. Soon thereafter, in 1966, Veale et al. described two families with multiple affected members, and Smilow et al. reported kindreds with three successive generations affected by juvenile polyps, suggesting an autosomal dominant inheritance [4, 5]. Sachatello et al. described another three-generation family with JP in 1970, where three affected members had both upper and lower GI polyposis, which they referred to as generalized JP [6]. Stemper et al. described a four-generation family with JP and multiple affected family members who had developed colon and upper GI cancers, providing further evidence for an autosomal dominant inheritance [7].

For a long time, little was known regarding the genetics of JP. It was known that congenital anomalies were seen in about 20% of cases, such as cardiac abnormalities and bowel malrotation [8]. Approximately 20–50% of the cases appeared to be familial, with the rest being sporadic [8–10]. Some studies have suggested a higher incidence in male patients [10–12], while others have found an equal incidence in males and females [13].

The first true linkage study in JP was somewhat limited, focusing upon markers near the *APC* gene in an Australian family, and linkage to this region was excluded [14]. The next genetic report in JP came from Jacoby et al. in 1997, which described a patient with colonic juvenile polyps, microcephaly, tricuspid insufficiency, and hypoplastic ears. An interstitial deletion was found by cytogenetic analysis at chromosome 10q22.1-q24.1, and examination of juvenile polyps from 13 unrelated JP patients and three with solitary juvenile polyps revealed loss of heterozygosity of the marker *D10S219* in 83% of polyps, consistent with a possible JP tumor suppressor gene at this locus [15]. This was the same region to which Nelen et al. had mapped a gene for Cowden syndrome (CS) to (10q22-23) in 1996 [16]. Based upon this finding, Marsh et al. genotyped 47 members of eight JP families with markers from this region, and found no evidence of linkage [17]. In 1997, Lynch et al. reported a mutation in the *PTEN* gene from chromosome 10q22 in a patient described as having both CS and JP (two family members with skin lesions and macrocephaly, one with colonic polyps and the other with small bowel cancer) [18]. In 1998, Olschwang et al. described mutations of *PTEN* in three JP families [19]. However, Eng et al. raised the question of whether the patients in both of these reports could have had CS [20]. Howe et al. found no evidence of linkage to chromosome 10q markers in a large JP kindred [21], and Marsh et al. did not find *PTEN* mutations in 14 familial and 11 sporadic JP cases [17]. *PTEN* mutations were also not found in 11 JP patients analyzed by Riggins et al [22]. These conflicting reports led to confusion for several years as to whether *PTEN* was the causative gene for both CS and JP, or that patients with hamartomatous polyps and *PTEN* mutations had just not yet manifested the defining features of CS. Later reports would fail to find *PTEN* mutation in JP cases, including the 24 examined by Woodford-Richens et al. [23] and the 35 by Howe et al. [24].

5.2 *SMAD4* Mutations in JP

Definitive evidence of a JP locus came in 1998, when the results of a linkage study by Howe et al. were reported [21]. They examined markers from candidate regions on chromosomes 2p (near *MSH2*), 3p (near *MLH1*), 5q (near *APC*), 10q (near *PTEN*), 12p (near *KRAS2*), 17p (near *TP53*), 18q (near *DCC*), and 19p (near *LKB1*) in a large Iowa JP kindred with 13 affected members (43 individuals total). Significant evidence of linkage (lod > 3.0) was found with seven markers from 18q21, with a maximum lod score of 5.00 at theta = 0.00 with the marker *D18S1099*. Critical recombinants in this family localized the gene to an 11.9 cM area that contained the tumor suppressor genes *DCC* and *SMAD4* (also known as *DPC4*). Sequencing analysis of both genes revealed no mutations in *DCC*, but a 4-bp deletion was found in exon 9 of *SMAD4* in all affected family members [25]. Four of eight other JP families studied in this report also were found to have germline *SMAD4* mutations, with three families sharing the same 1244_1247delACAG mutation. Several series later confirmed this finding, the first by Houlston et al., who found *SMAD4* mutations in only 1 of 13 familial and eight sporadic JP cases, and concluded that this gene was responsible for just a small fraction of JP cases (5%) [26]. However, a follow-up study from the same group later reported five *SMAD4* mutations in a total of 15 familial and nine sporadic JP cases (21% of cases) [23]. In 1999, Friedl et al. reported three *SMAD4* mutations in 11 JP cases, two of which were the same 4 bp deletion described earlier (1244_1247delACAG) [27]. Roth et al. found three *SMAD4* mutations in four familial and sporadic JP cases in 1999 [28], and Kim et al. reported mutations in three of five Korean JP cases in 2000 [29]. In a follow-up study looking at haplotypes of neighboring markers on chromosome 18q21 in four families sharing the same 1244_1247delACAG *SMAD4* mutation, Howe et al. concluded that these four families did not share a common ancestor and hypothesized that this deletion occurred at a mutational hotspot [30]. Pyatt et al. subsequently described 13 *SMAD4* mutations in 70 JP patients (18.6%), two of whom also had the 1244_1247delACAG mutation [31].

SMAD4 was originally identified by Hahn et al., after they noted that 90% of pancreatic cancers had deletions on chromosome 18q21.1 [32]. From this region, they found a gene encoding for a 552 amino acid peptide which they named *DPC4* (deleted in pancreatic cancer 4) [33]. Somatic mutations of this gene were frequently seen in pancreatic cancers, with homozygous losses seen in 30% of the cases, consistent with a tumor suppressor role. Somatic mutations were also seen in as many as 15–20% of sporadic colorectal tumors [34–37]. The name *DPC4* was replaced by *SMAD4* because of its homology to *Mad* genes in *Drosophila* and *sma* genes in *Caenorhabditis* [38]. The name *SMAD4* was later changed to *MADH4* by the HUGO gene nomenclature committee, then back to *SMAD4* once again (http://www.gene.ucl.ac.uk/nomenclature).

SMAD4 is a member of the TGF-b superfamily, which includes the TGF-b, activin, and bone morphogenetic protein (BMP) signaling pathways. These pathways are involved in a wide variety of processes in many different cell types, such as cell differentiation and embryogenesis, proliferation, and apoptosis. SMAD4 is the

common intracellular mediator involved in all the TGF-b superfamily pathways, whereas their specificities are determined by the cell-surface receptors that bind different superfamily ligands, and the coSMADS that associate with them. In general, the ligand binds to the plasma membrane serine/threonine kinase type II receptor, forming a complex, which then binds to the type-I receptor. This phosphorylates a glycine-serine rich domain of the type-I receptor, which then phosphorylates cytoplasmic coSMAD proteins (SMAD2 or SMAD3 for the TGF-b pathway; SMAD1, SMAD5, or SMAD8 for the BMP pathway). When phosphorylated, these coSMADs form oligomers that can then associate with SMAD4. The coSMAD/SMAD4 proteins then migrate into the nucleus, where a DNA binding protein joins them, forming a complex that can regulate the transcription of various genes through direct DNA binding to specific sequences. There are two other SMADs, SMAD6 and SMAD7, which inhibit these pathways by binding to the type I receptors, and interfering with phosphorylation [39]. Studies of other *SMAD* genes in JP patients have not revealed germline mutations, including the absence of *SMAD1*, *SMAD2*, *SMAD3*, or *SMAD5* mutations in 30 JP patients without *SMAD4* mutations in one study [40], and lack of *SMAD2*, *SMAD3*, or *SMAD7* mutations in four other JP patients [28].

The SMAD4 protein is divided into a Mad homology domain 1 (MH1), a linker region, and Mad homology region 2 (MH2). The MH1 domain is the DNA binding region of the protein, and most of the genes regulated by SMAD4 in the various pathways remain to be determined. The MH2 domain of SMAD4 is important in nuclear localization, coSMAD binding, and activation of transcription. This region is the most frequent target of mutations found in JP patients and in sporadic pancreatic cancers. Of the 26 different mutations reported in JP patients (15 deletions, two insertions, five nonsense, ten missense, and no splice site mutations), only one mapped to the MH1 domain of MADH4, five involved the linker region, and the rest occurred within the MH2 domain (Fig. 5.1) [24].

5.3 *BMPR1A* Mutations in JP

After careful study of a large number of JP families, it was clear that *SMAD4* mutations were only responsible for about 20% of JP cases. Therefore, Howe et al. [41] performed a genome screen for another JP locus using four families that did not have *SMAD4* and *PTEN* mutations by sequencing. Several positive lod scores were obtained with markers from chromosome 10q22-23, with a maximum lod score of 2.33 with *D10S573* (at theta = 0.10), which is in the region of the *PTEN* gene. Examination of genes known to map to this area in the genome database revealed that *BMPR1A*, the type I receptor for the BMP pathway, also mapped to this region. Two new simple tandem repeat markers close to *BMPR1A* were then developed, and these were respectively found to have lod scores of 4.74 and 4.17 at theta = 0.00 for both markers, confirming linkage of JP to this region. Sequencing of the *BMPR1A* gene in these four families revealed different truncating mutations in each which segregated in all affected members.

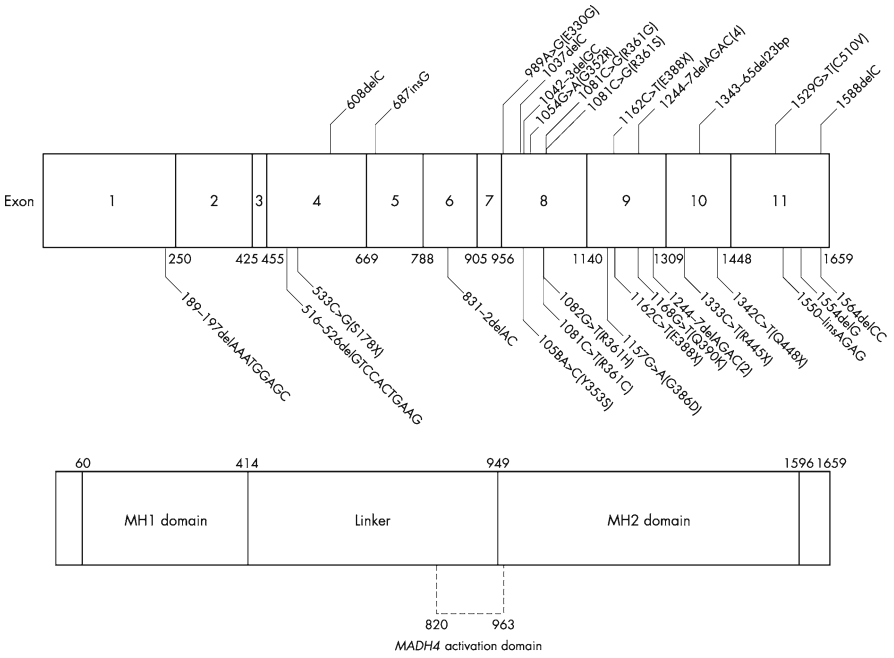

Fig. 5.1 Distribution of *SMAD4* mutations in juvenile polyposis patients. The *upper rectangles* represent the exons of the gene, with the different mutations shown above and below; when a numeral is present, this means that multiple cases have been found with that mutation (i.e., four patients for 1244-7delAGAC shown *above the rectangle*). The *lower rectangles* show the different domains seen in the protein (from Howe et al. [24], by permission of *Journal of Medical Genetics*)

These findings were later confirmed by Zhou et al., who found that 10 of 25 (40%) JP cases negative for *SMAD4* mutations had *BMPR1A* mutations [42]. In 2002, Friedl et al. reported five of 29 (17%) unselected JP cases to have germline *BMPR1A* mutations [43]. Howe et al. summarized their findings in 77 JP cases in 2004, where 16 (21%) had mutations of *BMPR1A*. Examining these cases and the others reported above, there were 31 separate mutations (nine deletions, one insertion, two splice sites, ten missense, and nine nonsense), which spanned eight of 11 exons. Approximately one-half of these involved the intracellular protein kinase region in exons 7 and 8, and the extracellular cysteine-rich domain was affected by another quarter of mutations (Fig. 5.2) [24]. A follow-up study by Pyatt et al. described eight *BMPR1A* mutations in 70 JP cases (11%) [31].

BMPR1A is the type I receptor for the bone morphogenetic protein pathway, originally described by Ten Dijke et al. in 1993 as an activin-like receptor with serine-threonine kinase activity [44]. Similar to the TGF-b pathway, BMPs bind to the type II receptors or to the type II and type I receptors together, then the type II receptor phosphorylates the type I receptor (*BMPR1A* or *BMPR1B*) [45].

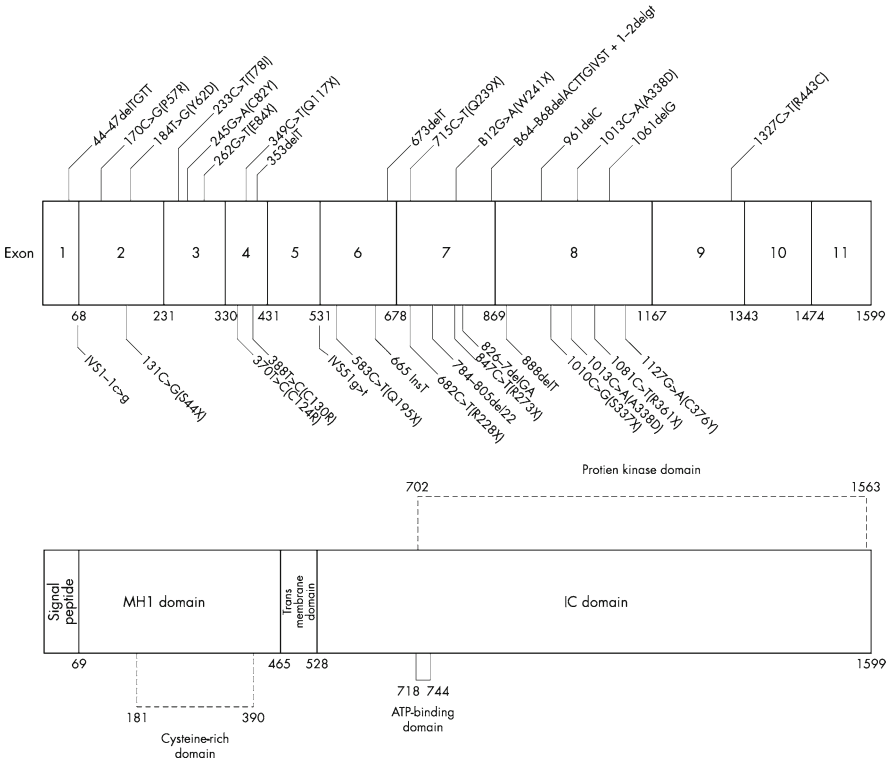

Fig. 5.2 Distribution of *BMPR1A* mutations in juvenile polyposis patients. The *upper rectangles* represent the exons of the gene, with the different mutations shown above and below. The *lower rectangles* show the different domains seen in the protein (from Howe et al. [24], by permission of *Journal of Medical Genetics*)

Intracellular SMAD1, SMAD5, and/or SMAD8 are phosphorylated by the type I/type II receptor complex, which may then bind to intracellular SMAD4. The SMAD4/coSMAD complex may next enter the nucleus to regulate gene transcription [39]. The BMP pathway is involved in the regulation of chondrogenesis, osteogenesis, the developing mesoderm, extracellular matrix production, and epithelial/mesenchymal cell interactions with relation to morphogenesis [46].

Other researchers have reported germline mutations in JP patients. Kim et al. evaluated the same group of five Korean patients in whom they found three with *SMAD4* mutations [29], and found one (without *SMAD4* mutation) with a *BMPR1A* missense mutation [47]. Delnatte et al. studied four patients with a particularly severe form of JP (JP of infancy), with both upper and lower GI polyps, and macrocephaly, and determined they had contiguous 10q23 deletion encompassing both *BMPR1A* and *PTEN* genes [48]. Salviati et al. reported a patient with JP and a larger deletion of 10q22-23 (approximately 12 Mb, vs. 2 Mb in two of Delnatte's patients,

and 1.2 Mb in another) encompassing *BMPR1A* and *PTEN* who had a milder JP phenotype, suggesting that patients who have lost these two genes may have a phenotype other than the characteristically more virulent form JP of infancy [49].

Only a few studies have examined genotype–phenotype correlations. Sayed et al. reported that 17 of 19 (89%) *SMAD4* and *BMPR1A* mutation positive (MUT+) and 13 of 25 (52%) MUT cases had a family history of GI cancer (these differences were significant at $p=0.01$). The prevalence of upper GI juvenile polyps in families was 86% in *SMAD4* mutation-positive JP patients, 10% in *BMPR1A* mutation cases, and 23% in mutation-negative cases [50]. A similar report from Friedl et al. demonstrated that four of seven *SMAD4* mutation-positive cases had gastric polyposis, which was only seen in one of five cases with *BMPR1A* mutations, and two of 17 mutation-negative patients [43]. These studies suggest that JP patients with *SMAD4* mutations are more likely to have generalized JP (with gastric involvement), while those without these mutations are more likely to have JP coli.

Sweet et al. found two cases of germline *BMPR1A* mutation in 49 cases of unexplained hamartomatous polyposis [51]. In this paper, they described two of 14 JP patients with missense mutations of *ENG* [51], a gene previously found to predispose to hereditary hemorrhagic telengiectasia (HHT) [52]. Although Sweet et al. suggested that this may represent a new JP gene, a follow-up study by Howe et al. examining 31 cases of *SMAD4*-, *PTEN*-, and *BMPR1A*-mutation-negative JP patients provided no support for this contention; they found four silent polymorphisms and two missense mutations which had been previously reported to be polymorphisms in HHT patients, but no clear demonstration of disease-causing mutations [53]. Evidence of a genetic association between JP and HHT was reported by Gallione et al. in 2004, in which the authors described the results of sequencing *ENG*, *ACVR1* (another HHT gene), and *SMAD4* in seven patients with the clinical manifestations of both JP and HHT. All seven had *SMAD4* mutations (three frameshifts, four missense), and the authors suggested that JP patients with *SMAD4* mutations be checked for features of HHT (arteriovenous malformations, mucocutaneous telangiectases, digital clubbing, osteoarthropathy, hepatic arteriovenous malformations, and cerebellar cavernous hemangioma) [54]. Gallione et al. later studied 30 HHT patients with no signs of JP for *SMAD4* mutations, who were negative for *ENG* and *ACVR1* mutations, and found that three (10%) had *SMAD4* germline mutations. They suggested that HHT patients be sequenced for *SMAD4* in addition to the two known HHT genes (*ENG* and *ACVR1*), and patients with these mutations be screened for GI polyps and cancer [55].

In summary, from the studies listed above, there are currently two JP genes that have been identified by both linkage and observing segregation of mutations in affected members of JP families. Both *SMAD4* and *BMPR1A* fulfill these criteria, and each account for approximately 20% of JP cases. *PTEN* mutations are probably diagnostic for CS rather than JP in patients with hamartomatous polyps, and it seems unlikely that *ENG* is a JP predisposition gene. There might be further uncharacterized genetic heterogeneity in JP and new genes awaiting discovery.

5.4 Cowden Syndrome

Lloyd and Dennis named this syndrome in 1963 after the proband of a family, [56] and Weary et al. coined the term multiple hamartoma syndrome in their description of five cases, highlighting the contribution of ectoderm, mesoderm, and endoderm to the characteristic lesions of CS. They suggested an autosomal dominant mode of inheritance [57], as did Gentry et al., who described seven affected members in three generations. Benign mucocutaneous lesions, thyroid lesions, benign and malignant breast tumors, and gastrointestinal polyps were seen [58]. A slight female predisposition has been reported in some studies [59–61], but this could also reflect the fact that females are more commonly diagnosed because of the high incidence of breast lesions in CS.

The International Cowden consortium has established pathognomonic (six facial papules, three of which are trichilemmomas; oral papilomatous; six palmoplantar keratoses), major (breast cancer, thyroid cancer, macrocephaly, LDD) and minor (goiter, benign breast lesions, hamartomatous GI polyps, mental retardation, lipomas, fibromas, and GU abnormalities) diagnostic criteria for CS [62]. If there are no pathognomonic criteria, then the diagnosis is made by two major criteria, one major and three minor, or four minor criteria. The association of colorectal cancer with CS is weak, with Starink et al. describing only three cases in 100 CS patients [61].

5.5 *PTEN* Mutations in CS

The genetic basis of CS was poorly understood until 1996, when Nelen et al. performed a linkage-based genome screen of 12 CS families, comprising 40 affected individuals. They found significant evidence for linkage (lod scores > 3.0) with eight markers from chromosome 10q22-23, with a maximum lod of 8.92 (theta = 0.02) with *D10S573*, and no evidence of genetic heterogeneity [16]. At that time, there were no good candidate genes known to map to this region, but in 1997 three groups described a tumor suppressor gene mapping to 10q23 [63–65]. Li et al. performed deletion mapping in breast tumors and identified a gene encoding for a 403 amino acid protein in the common region of deletion. This gene had a protein tyrosine phosphatase domain, homologous to the chicken protein tensin and bovine protein auxilin [64]. They named the gene *PTEN*, the P standing for phosphatase, and TEN having a dual meaning of being homologous to tensin and being from chromosome 10. They found truncating mutations in two of 20 breast cancers, three of six glioblastomas, and one prostate cancer cell line (another had a missense mutation), consistent with a possible tumor suppressor role [64]. The same year, Steck et al. performed deletion mapping in glioblastoma cell lines and identified a gene mapping to the common area of deletion by exon trapping [65]. They found mutations in seven of 17 glioma cell lines, six of 26 primary gliomas, two of 14

breast cancers, and one of four kidney tumors. They named the gene *MMAC1*, for mutated in multiple advanced cancers. Li and Sun followed with their report of isolating a protein tyrosine phosphatase EST whose cDNA turned out to be the same as *PTEN/MMAC1*, which they named *TEP1*, for TGF-b regulated and epithelial cell-enriched phosphatase. They noted that the protein structure shared homology with dual specificity kinases, and to the proteins tensin (which binds actin) and auxilin (which is involved in vesicular transport). Its expression was reduced by treatment with TGF-b, and the coding sequence was the same as the recently described *PTEN/MMAC1* [63].

Shortly thereafter, Liaw et al. sequenced most exons of *PTEN* (the intron boundaries of exons 2, 3, and 4 were difficult to characterize) in five CS families, and found four with germline mutations [66]. In each case, the mutation segregated with the CS phenotype in the families, each of which had two to four affected individuals. Two families shared the same Gly129 to Glu129 missense mutation in exon 5, and these and a Glu157Stop nonsense mutation were predicted to disrupt phosphatase activity. The Arg233Stop nonsense mutation found in exon 7 of another family was believed to potentially disrupt the tertiary structure of the protein. The authors speculated that since hamartomas are the hallmark of CS that perhaps PTEN functions to help guide intracellular interactions, which become disorganized when there are mutations.

Nelen et al. then described eight familial and 11 sporadic cases of CS sequenced for *PTEN* mutations, and found mutations in nine patients (two familial, seven sporadic cases) [67]. These consisted of one deletion, two insertions, one splice site mutation, three nonsense mutations, and two missense mutations. One exon 5 mutation (Arg130Stop) was seen in two cases, and five of the nine mutations affected this exon important for phosphatase activity. In the two familial cases, the mutation segregated with the affected phenotype in each generation. Four patients also had Lhermitte–Duclos disease (LDD), characterized by cerebellar gangliocytoma and megalencephaly.

PTEN is a dual specificity phosphatase, which means it dephosphorylates not only tyrosine, but also serine and threonine residues. It has activity on both protein and lipid substrates [68]. *PTEN* has roles in the cell cycle, apoptosis, embryogenesis, and neoplasia. One function relates to its involvement in the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB)/antiapoptotic serine threonine kinase (AKT) pathway. Here, PTEN-mediated dephosphorylation of the phosphatidylinositol (3,4,5) triphosphate (PIP3) substrate keeps cell growth and survival in check, while up-regulation of PI3K-PKB/AKT promotes growth. When PIP3 is reduced, there is less PKB/AKT in the cell membrane, which may lead to G1 cell cycle arrest and apoptosis [68]. PTEN may also be involved in regulation of the mitogen-activated kinase (MAPK) pathway, which also plays a role in cell differentiation and growth [69]. The tumor suppressor role of *PTEN* therefore is mediated by this dephosphorylative activity that inhibits cell proliferation, which is lost in the presence of inactivating mutations, as described in CS.

In a follow-up to the Liaw et al. paper, Marsh et al. examined a total of 37 CS families for *PTEN* mutations [70]. Thirty (81%) cases were found with mutations

(Fig. 5.3), of which six (20%) were missense, nine (30%) were nonsense, four (13%) were deletions, six (20%) insertions, three (10%) were splice site mutations, and one was a deletion/insertion. No mutations were found in exons 1, 4, or 9, while 43% of mutations involved exon 5. Six (20%) of the mutations were found in exon 7, and four (13%) in exon 8, which both contain protein tyrosine kinase phosphorylation sites. Furthermore, they found an association between *PTEN* mutation and breast disease. In 27 mutation-positive CS families, 12 had breast cancers and eight had benign breast disease. In seven mutation-negative cases, one had malignant and six had benign breast lesions. The difference in malignant breast disease in *PTEN* mutation-positive versus negative cases nearly reached statistical significance ($p=0.08$). Marsh et al. also examined 64 cases with a CS-like phenotype who did not meet the consortium criteria for CS, which require meeting major (breast cancer, thyroid cancer, macrocephaly, LDD, trichilemmomas, or mucocutaneous papules) and minor (goiter, benign breast lesions, hamartomatous GI polyps, mental retardation, lipomas, fibromas, and GU abnormalities) pathognomonic criteria [71]. Only one *PTEN* mutation was found, in a patient with thyroid cancer whose mother had breast and endometrial cancer, but neither had skin lesions or macrocephaly. They concluded that *PTEN* only plays a minor role in families that do not meet the criteria for CS [72]. In a follow-up study of 97 CS cases where *PTEN* mutations were not identified, Zhou et al. detected nucleotide changes in the promoter region of *PTEN* in nine (9.3%) patients, thereby increasing the overall prevalence of *PTEN* genetic changes to 90% (from 81%) in CS patients [68].

Nelen et al. examined *PTEN* in seven familial and six sporadic Dutch CS patients, and found eight (five familial, three sporadic) with mutations. These consisted of microdeletions in exons 2 and 8, three splice-site mutations, nonsense mutations in exons 5 and 8, and a missense mutation in exon 6. Combined with their previous study, seven of nine familial cases had *PTEN* mutation, as did 10 of 13 sporadic cases. They looked for genotype–phenotype correlations, but could not make statistically significant associations. They did find that LDD patients did not have missense mutations. Although Marsh et al. suggested that CS patients with breast disease are more likely to have mutations [70], Nelen did not find this, and in fact described that four of his five CS patients without *PTEN* mutation developed breast cancer, as did the brother of one of these patients [73]. They were able to estimate the prevalence of CS in the Netherlands to be between one in 200,000–250,000.

5.6 Bannayan–Riley–Ruvalcaba Syndrome

Bannayan–Riley–Ruvalcaba syndrome (BRRS) was first described by Riley and Smith in 1960, in which the mother and four of her seven children had macrocephaly and pseudopapilledema with no mental retardation [74]. Bannayan described a patient with macrocephaly, lipomatosis, and angiomatosis in 1971 [75], and Zonana et al. suggested an autosomal dominant inheritance in 1976 by describing this condition in a father and two of his sons [76]. Ruvalcaba et al. described

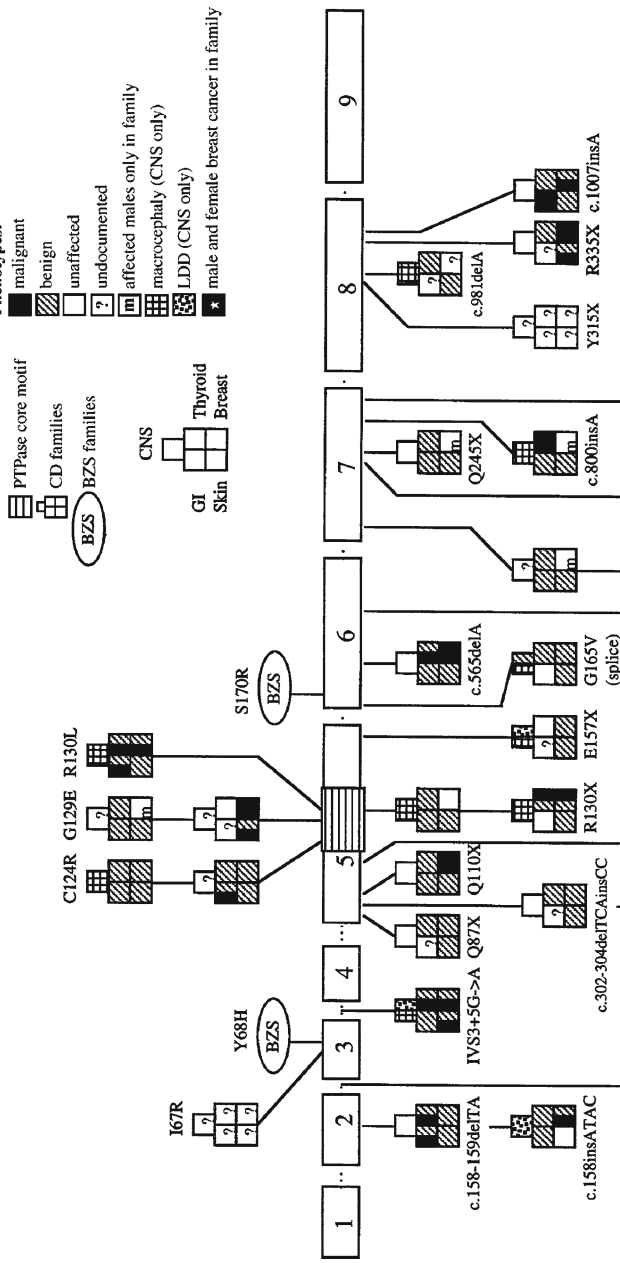

Fig. 5.3 Mutations and phenotypes of CS patients with *PTEN* mutations. The *rectangles* represent each exon of *PTEN*, and the phenotypes are shown in the key to the upper right (from Marsh et al. [70], by permission of *Human Molecular Genetics*)

some of the other features now known to be common in BRRS in 1980, when he reported two cases with macrocephaly, pigmented lesions of the penis, and intestinal polyps [77]. Gorlin et al. helped to clarify the phenotype of BRRS with their description of a family with 12 affected members and showed that the previously named syndromes Ruvalcaba–Myhre, Riley–Smith, and Bannayan–Zonana were of similar conditions [78], supporting Cohen's renaming them as Bannayan–Riley–Ruvalcaba syndrome in 1990 [79].

Since these reports, it has become clear that distinguishing between BRRS and CS can be difficult. Fargnoli et al. reported a patient with macrocephaly, lipomas, thyroid adenoma, colonic polyps, and hyperpigmented macules of the penis. These findings were consistent with BRRS, but also met the diagnostic criteria for CS [80].

The genetics of BRRS were clarified in 1997. Zigman et al. reported two BRRS patients with deletions from 10q23.2-q24.1, with microsatellite markers showing overlap with the putative region of the CS gene [81]. Marsh et al. sequenced *PTEN* in two BRRS families, and found an S170R mutation (exon 6) in one, and R233X mutation (exon 7) in the other (which had also been seen in a CS family [66]). They concluded that BRRS and CS may be allelic disorders [82]. A follow-up study from this group revealed *PTEN* germline mutation in four of seven (57%) unrelated BRRS families [70]. The largest series was an update by Marsh et al. in 1999 encompassing 43 BRRS cases (16 sporadic and 27 familial, with 11 families having features of both BRSS and CS) [83]. *PTEN* mutations were found in 26 of 43 cases (60%), and there was a correlation between *PTEN* mutations, breast cancer, and fibroadenomas, as well as lipomas, but not with familial versus sporadic cases. No cases of colorectal adenocarcinoma were seen in these BRRS patients.

5.7 Peutz–Jeghers Syndrome

The first description of a Peutz–Jeghers syndrome (PJS) patient was likely reported in 1895, in which Conner described 12-year-old twins with pigmented lesions of the lips and mouth, who appeared anemic [84]. One twin died at age 20 from bowel obstruction, and the other of breast cancer at age 52 [85]. In 1921, Peutz linked the characteristic features of mucocutaneous pigmentation with intestinal polyps in a description of a three-generation family with seven affected members [86]. In 1949, Jeghers et al. described the clinical features seen in ten PJS patients, and recognized its autosomal dominant inheritance [85]. In 1954, Bruwer et al. coined the name Peutz–Jeghers syndrome [87]. In 1997, comparative genomic hybridization was used by Hemminki and colleagues to examine epithelium-enriched DNA from 16 hamartomatous intestinal polyps and normal tissue from a single PJS patient to look for areas of the genome with different signal intensity, on the basis that a tumor suppressor gene might have somatic loss in relatively few areas. They found loss of 19p in six of 16 polyps, which was confirmed by loss of heterozygosity studies using microsatellite markers. This was followed up with linkage studies in 12 PJS families with markers from 19q, resulting in a maximum lod score of 5.40 at

theta = 0.00 with the marker *D19S886*, and a multipoint lod score of 7.00 at *D19S886*. There was no suggestion of genetic heterogeneity, thus providing strong evidence of a single PJS gene on chromosome 19p [88].

The finding of linkage to markers on 19p was quickly confirmed by Amos et al., who studied five PJS families by linkage and found a two-point lod score of 4.45 and multipoint lod score of 7.52 at theta = 0.00 with *D19S886*. This was the most telomeric marker tested, residing within the last 3 Mb of the chromosome [89]. Also in 1997, Mehenni et al. confirmed linkage in six PJS families to *D19S886*, with a two-point lod score of 4.74 at theta = 0.045 and a multipoint lod score of 7.51. In this same group of families, a second PJS locus was suggested on 19q13.4 with the finding of a lod score of 3.80 at theta = 0.13 with the marker *D19S880* [90]. Olschwang et al. examined 20 PJS families for 19p linkage and found that 17 had haplotypes consistent with 19p linkage, while three appeared to have recombination events and to be unlinked. The maximum lod score found, however, was only 1.45 at theta = 0.10 with *D19S886* [91].

5.8 *LKB1* Mutations in PJS Patients

In 1998, two groups reported finding the putative PJS gene. Hemminki et al. screened a cosmid contig spanning the 800 kb region defined by critical recombinants between the markers *D19S886* and *D19S883* using two cDNA libraries. Twenty-seven transcripts were identified, of which one had homology to a serine threonine protein kinase that corresponded to an EST named *LKB1* that encoded for a 433 amino acid protein. This had 84% homology to the *Xenopus XEEK1* serine/threonine kinase, and was subsequently found to be expressed in all human tissues examined. This candidate gene was sequenced in 12 PJS families that appeared to be linked to chromosome 19p, and 11 different mutations were found (four nonsense, one missense, one insertion, and five deletions, two in frame and three truncating). These mutations were thought to result in decreased kinase activity, and it was suggested that this was the first description of loss of kinase activity in causing human disease, in contrast to the activation seen of *RET* in MEN2 and *CDKN2* in familial melanoma [92].

Jenne et al. also evaluated genes in the vicinity of *D18S886*, including a novel serine-threonine kinase gene with nine exons, which they called *STK11* (which was the same gene as *LKB1*) [93]. Of five PJS patients sequenced, one had an inversion/deletion between exons 4 and 7 (which was also seen in two other affected family members), two had deletions and frameshifts, one had a splice-site mutation leading to exon 4 skipping, and another had a nonsense mutation.

A follow-up study by Gruber et al. included Jeghers' original family and five others that were consistent with 19p13 linkage. All were found to have *STK11* mutations. Further analysis revealed loss of the normal allele in 11 of 12 hamartomas or adenocarcinomas from four PJS patients, suggesting that the PJS gene is consistent with tumor suppressor function, and one that may occur early in the process of tumor progression [94].

Nakagawa et al. examined 15 Caucasian and Japanese PJS families for *STK11* mutations, and found changes in ten families. Two had presumed splice-site changes, three had insertions with frameshifts, one had a 3-bp deletion, three others had deletion with frameshift, and one had a nonsense mutation. Three changes occurred in a mononucleotide repeat (CCCCCC) in codons 279–281 in exon 6, which appears to be a mutational hotspot [95].

Westerman et al. examined *LKB1*/*STK11* mutations in 19 PJS families, and found 12 novel mutations for an overall rate of 63%, raising the question of genetic heterogeneity. Nine mutations predicted for a truncated protein (three deletions, three insertions, two splice-site, one nonsense mutation), and three were missense mutations. Five of the 12 families appeared to have de novo mutations, and segregation of mutations was unclear in two families, demonstrating that sporadic cases of PJS are common [96].

Wang et al. evaluated nine familial and three sporadic PJS cases for *LKB1* mutation, and seven (58%) novel mutations were found (two nonsense, one missense mutation, and four deletions). Of the five families without mutations, four still had linkage results compatible with 19p13, and therefore other alterations, such as promoter changes or large deletions of *LKB1*, could have been at work but not detected by sequencing [97].

Miyaki et al. looked for changes in both the germline and in polyps from PJS patients. They found germline mutations in six families (67%), five with frameshifts (three deletions, two insertions) and one with a splice site mutation. A total of 19 of 27 (70%) polyps had somatic changes consistent with inactivation of the wild-type allele. Of these, 14 had LOH of 19p microsatellite markers, while five had truncating mutations of *LKB1* (four of five with the same 4-bp deletion of exon 6). They found mutations of b-catenin in six polyps, and 19p LOH, b-catenin mutation, and p53 mutation in one cancer. They felt that this progression supported a hamartoma–adenoma–carcinoma sequence [98]. Gruber et al. came to similar conclusions through the finding that 11 of 12 hamartomas and adenocarcinomas had 19p LOH, and suggested that *STK11* was a gatekeeper involved in the formation of hamartomatous polyps in PJS prior to adenocarcinoma [94].

Some studies have found relatively low proportions of PJS patients with *LKB1*/*STK11* mutations, suggesting that there may indeed be other PJS genes that have not yet been found. Boardman et al. looked for *LKB1* mutations by conformation sensitive gel electrophoresis (CSGE) in ten familial and 23 sporadic PJS cases. They found only two (20%) mutations in the familial cases (891C>T/A297S and 844insC) and four mutations in sporadic cases (17%) [99]. Lim et al. found mutations in 17 of 33 JPS families (52%) using CSGE [100], raising the question of whether this technique just has lower sensitivity than studies that use primarily DNA sequencing, or that there was significant genetic heterogeneity in these studies. Scott et al. examined 14 unrelated Australian PJS probands (five with a family history) by DHPLC, and found mutations in only seven (50%; three deletions, two splice-site mutations, two missense mutations). Whether this was due to use of DHPLC for screening instead of sequencing or genetic heterogeneity is not known [101]. Jiang et al. evaluated ten unrelated PJS cases both by sequencing and

by protein truncation test, and found germline *STK11* in only one patient. The thoroughness of this study certainly suggests that there is genetic heterogeneity, or other means of gene inactivation, such as promoter methylation or changes in other regulatory regions [102].

Along these lines, Esteller et al. examined the *LKB1* promoter by methylation-specific PCR to determine the role of epigenetic inactivation in a variety of tumors. They evaluated 15 colon, 11 lung, seven ovary, five breast, three thyroid, three brain, three prostate, three leukemia, and one cervical cancer cell lines, and 195 primary tumors. Three colorectal cancer cell lines and one cervical cancer cell line were methylated, as were one primary colorectal cancer and three testicular tumors. Five of 11 uncommon papillary subtype breast tumors showed methylation, as did four of 22 hamartomatous polyps derived from three PJS patients. These data demonstrate that *LKB1* promoter methylation may be another means of gene inactivation [103].

Another explanation for the lack of detection of *LKB1*/*STK11* mutations in a significant subset of PJS families rather than genetic heterogeneity could be large-scale deletions of the gene. Le Meur et al. described a patient with pathognomonic features of both neurofibromatosis and PJS in 2004, who harbored a 220–250 kb deletion encompassing the *STK11* gene [104]. Aretz et al. sequenced *LKB1* in 71 cases presumed to have PJS (56 meeting clinical criteria, 12 with mucocutaneous pigmentation or PJS polyps, three with no available records), and found mutations in 37 (52%). The remaining 34 cases without mutations were then examined for deletions of *LKB1* by multiplex ligation-dependent probe amplification, and 17 were found. Four had mutation of the entire gene (including the promoter and non-coding exon 10), seven involved the promoter and exon 1, two had deletion of exons 2–10, and others involved exons 2–3, exons 4–5, exon 8 only, and at least 0.9 Mb upstream of *STK11* into exon 1. The overall rate of *STK11* mutation or deletion in the subset of PJS patients meeting clinical criteria was 94%, and the authors questioned whether there really was another PJS gene [105].

In 1998, Mehenni et al. demonstrated that four LKB1 mutant proteins (L67P, K78I, D176N, and W308C) had lost their protein kinase activity as assessed by autophosphorylation, which they believed was responsible for the development of PJS [106]. Boudeau et al. later examined the functionality of different *LKB1* mutations found in Italian PJS patients when cloned into a CMV expression vector. Two of these were missense mutations (R304W, I177N) and four were deletions. All six failed to autophosphorylate at the major site in *LKB1* (Thr336), or to phosphorylate p53. Five of the six isoforms remained localized to the nucleus, in contrast to the cytoplasmic and nuclear localization of wild-type *LKB1*, nor were any of the mutant isoforms capable of suppressing the growth of the G361 melanoma cell line, in contrast to wild-type *LKB1*. These results support that these mutations result in loss of kinase function and that loss of this activity may be the cause of PJS [107].

In a study of 39 polyps and five carcinomas from PJS patients (17 from 13 families), Entius et al. looked for LOH on 19p13 (*LKB1*), 5q21 (*APC*), 17p13 (*p53*), and 18q21-22 (*MADH2*, *MADH4*). All of the carcinomas had LOH on 19p13, as did 15

of 39 (38%) polyps. A higher proportion of LOH was seen in polyps from patients with cancer (six of seven, 86%) than those without (nine of 31, 29%; $p = 0.01$). Four of five cancers had *APC* mutations, but no polyps or cancers had 5q21 LOH. No tumors had 17p13 LOH, and only one cancer had 18q LOH, and by immunohistochemistry all had p53 and SMAD4 expression. This study suggested that cancer progression in PJS patients involved inactivation of *LKB1*, but not of other genes commonly involved in sporadic colorectal carcinoma [108].

5.9 PJS Genotype–Phenotype Associations

A few groups have evaluated the correlation of *STK11* genotype with phenotype. Amos et al. sequenced 33 PJS patients for *STK11*, and found mutations in 22 (69%), of which 27% were insertions, 27% were missense mutations, 18% were deletions, 18% were nonsense mutations, and 5% were within splice-sites. Patients with missense mutations had onset of GI symptoms and first polypectomy (21 and 28 years, respectively) at older ages than those with truncating mutations (10 years and 18 years). The median age of onset of gastric polyps was also older in patients with missense (23 years) versus truncating mutations (13 years) [109].

Schumacher et al. examined 41 PJS cases for *STK11* mutation by SSCP, and found that 27 (66%) had germline mutations, then combined these patients with 105 others described in the literature between 1998 and 2004 to determine the risk of specific mutations on developing cancer. They found that patients with breast cancer had truncating mutations more frequently than missense mutations, and that PJS patients with any cancer more commonly had truncating mutations or missense mutations in the C-terminus [110]. Lim et al. reported an increased risk of GI and breast cancers in the 17 of 33 (52%) PJS families with *LKB1* mutations, with stan-

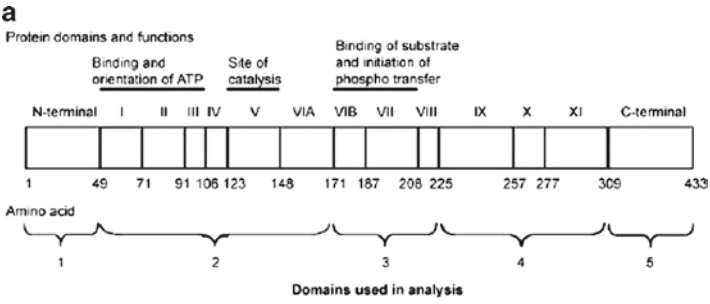

Fig. 5.4 Mutations found in *LKB1/STK11* in PJS patients. (**a**) Functional domains of the protein, with kinase domains I–XI (*gray*). (**b**) Mutations found in PJS patients with *rectangles* designating predicted proteins, and *dark gray* portions the kinase domains (from Hearle et al. [111], by permission of *Clinical Cancer Research*)

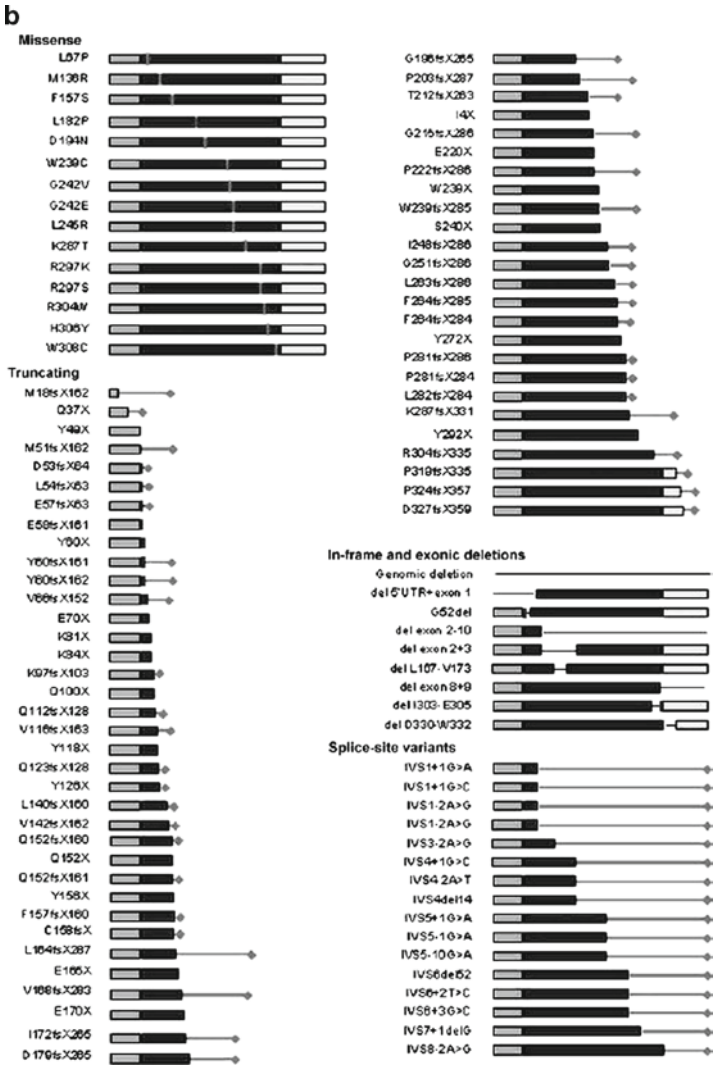

Fig. 5.4 (continued)

dardized mortality ratios of 32 for GI cancer (25 for JPS patients without *LKB1* mutations) and 13.9 for breast cancer, compared to the normal population [100].

A much larger study by Hearle et al. pooled 419 patients meeting the diagnostic criteria for PJS from Europe, Australia, and the United States (Fig. 5.4). Two hundred ninety-seven (70.1%) had mutations of *STK11/LKB1*, and 96 tumors were confirmed in the group (11 patients had two tumors, none had more than two). Overall the risk of developing cancer was 85% by age 70, fourfold higher than the population risk of 18%. The risk of developing GI cancer by age 70 was 57%,

breast cancer 45% and gynecologic cancer 18% (for females), lung cancer 17%, and pancreatic cancer 11%. There was no difference in the overall risk of cancer or of developing GI or breast cancers in PJS mutation carriers versus PJS patients found not to have mutations ($p=0.43$, $p=0.47$, and $p=0.77$, respectively). When mutations were divided into truncating and nontruncating, there was a higher risk of all cancers ($p=0.12$), of GI cancer ($p=0.09$), and female breast cancer ($p=0.49$) in the truncating group, but these differences did not reach statistical significance. When analyzed according to five different domains in *STK11*/*LKB1*, there did not seem to be any significant differences in cancer incidence, but the numbers were too small to make meaningful comparisons [111].

5.10 Mouse Models of PJS

Miyoshi et al. described an *Lkb1* knockout mouse in 2002, where exons 2–4 were deleted by homologous recombination. When heterozygotes were crossed, there were no *Lkb1*(−/−) progeny, suggesting that biallelic inactivation was embryonically lethal. Heterozygous *Lkb1*(+/−) mice developed gastric polyps (93% by 20 weeks, and 100% by 40 weeks of age), and 31% developed small intestinal polyps by age >50 weeks. These polyps were hamartomatous with arborizing smooth muscle within the lamina propria, similar to PJS polyps in humans. Furthermore, these polyps retained both the wild-type and mutant *Lkb1* alleles, demonstrating that loss of the normal allele was not required for the development of polyps. Since Lkb1 protein expression was half that seen in wild-type mice, it appeared that haploinsufficiency alone was enough to cause polyps. Interestingly, most heterozygous mutant mice developed hepatocellular carcinoma but no other extraintestinal manifestations [112]. Katajisto et al. created a conditional *Stk11* knockout in GI smooth muscle cells, which led to premature death of *Stk11*(+/−) mice at 12.5 months [113]. These mice had PJS-type polyps and defective mesenchymal TGF-b signaling, which normally inhibits epithelial proliferation.

5.11 Summary

Causative genes have been identified for most of the hamartomatous polyposis syndromes, making genetic testing of at-risk individuals possible. Furthermore, these genes may be useful for the molecular classification of polyposis patients when the pathognomonic features of a certain syndrome are absent. This will allow clinicians to more appropriately screen for and counsel patients regarding other associated anomalies commonly seen with each syndrome, which otherwise might not have been considered. Much work remains, however, in understanding how these germline mutations result in polyps, and what further changes are involved in the progression to cancer.

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Chapter 6
Hyperplastic Polyposis Syndrome: Colorectal Cancer Predisposition

Joanne Young

Abstract A major paradigm shift in the way we view the initiation and progression of colorectal cancer (CRC) has occurred within the last decade. For many years, the malignant transformation of adenomatous polyps (adenomas) was considered to be the only route to cancer in the human colon. The other common polyp type, with serrated but non-dysplastic features (also referred to as hyperplastic or metaplastic polyps) was seen as innocuous. However, with the recognition at a molecular level that some serrated polyps may act as the precursor lesions for CRCs, the "serrated pathway" came of age, and has provided an alternative mechanism for the development of CRC, existing alongside the traditional adenoma–carcinoma sequence. Molecular evidence for the malignant transformation of serrated polyps was first observed in a colorectal cancer patient with a condition called hyperplastic polyposis syndrome (HPS), where multiple serrated polyps are present throughout the colon. Since this pivotal event, HPS has served as a molecular model for the serrated pathway of CRC development, analogous to that provided by FAP for the sporadic adenoma–carcinoma sequence. In this chapter, the evidence that individuals with HPS have a genetic predisposition, which increases their risk of developing CRC, will be examined. Though the genetic lesion underlying HPS is yet to be identified, its inclusion as a genetic CRC predisposition is a concept whose time has come.

Keywords Hyperplastic polyposis • Serrated polyps • BRAF mutation • CpG island methylator phenotype • Familial cancer predisposition

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6.1 Introduction

A major paradigm shift in the way we view the initiation and progression of colorectal cancer (CRC) has occurred within the last decade. For many years, the malignant transformation of adenomatous polyps (adenomas) was considered to be the only route to cancer in the human colon [1]. The other common polyp type, with serrated but non-dysplastic features (also referred to as hyperplastic or metaplastic polyps) was seen as innocuous. However, with the recognition at a molecular level that some serrated polyps may act as the precursor lesions for CRCs, the "serrated pathway" came of age [2], and has provided an alternative mechanism for the development of CRC, existing alongside the traditional adenoma–carcinoma sequence [3].

Though histological observations suggesting that at least some serrated polyps may develop features associated with malignancy have been in the literature since the late 1970s [4, 5], molecular evidence for the malignant transformation of serrated polyps was first observed in a colorectal cancer patient with a condition called hyperplastic polyposis syndrome (HPS) [6], where multiple serrated polyps are present throughout the colon. Since this pivotal event, HPS has served as a molecular model for the serrated pathway of CRC development, analogous to that provided by FAP for the sporadic adenoma–carcinoma sequence [7]. The recognition of the serrated pathway has provided the opportunity to further explore the remaining unexplained portion of familial CRC [8, 9]. In this chapter, the evidence that individuals with HPS have a genetic predisposition, which increases their risk of developing CRC, will be examined. Though the genetic lesion underlying HPS is yet to be identified, its inclusion as a genetic CRC predisposition is a concept whose time has come [10].

6.2 Hyperplastic Polyposis Syndrome

The two major types of epithelial polyps in the human colorectum are the adenoma and the serrated polyp. Serrated polyps is a general term encompassing all polyps with serrated glandular architecture [11]. Serrated polyps are frequently observed in aging populations, with a prevalence of 5–11% in individuals undergoing autopsy [12]. The vast majority of these polyps are diminutive lesions with negligible malignant potential and are situated in the distal colon [13]. [For a detailed review of the serrated polyp sub-types in the human colorectum, the reader is referred to Chap. 9.] However, serrated polyps are also seen in a condition called hyperplastic polyposis syndrome (HPS), variously known as serrated adenomatous polyposis [14] or hyperplastic polyposis coli syndrome [15], which was first described in 1977 by Spjut and Estrada [16]. In HPS, the serrated polyps demonstrate features that set them apart from the bulk of common serrated polyps in that they may be unusually numerous or large, and may exhibit atypical histological architecture. It is of clinical importance to distinguish HPS from the diminutive serrated non-dysplastic polyps that occur in the distal colon in older patients.

HPS has been phenotypically defined by Burt and Jass as (1) at least five histologically diagnosed hyperplastic polyps proximal to the sigmoid colon, two of which are greater than 10 mm in diameter OR (2) any number of hyperplastic polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with hyperplastic polyposis OR (3) more than 30 hyperplastic polyps of any size but distributed throughout the colon [17]. Several investigators have suggested that hyperplastic polyp numbers diagnostic for HPS be reduced to 20 [18] and 10 [19] respectively. In addition, Higuchi and Jass have suggested that atypical serrated polyps, including sessile serrated adenomas (SSAs), polypoid serrated adenomas and mixed polyps, are counted in the total and that the polyp count can be cumulative over time [20]. Polyposis in HPS is frequently pan-colonic [17, 21]; however, polyps may be concentrated in either the distal or proximal colon [19]. Described in earlier reports in order to distinguish it from FAP, HPS was originally considered to have no significant clinical consequences [22]. Most cases of HPS present in the sixth or seventh decades of life [15, 23], but the disorder may be apparent considerably earlier [14, 24–26]. HPS is more common in Europeans [21], shows evidence of genetic predisposition [10], and importantly, is a condition now considered to be associated with a high risk of CRC [15]. The features of HPS are shown in Fig. 6.1.

HPS is relatively rare. Rubio and colleagues reported that only ten cases were observed in a 1026-bed Scandinavian Hospital over a 16-year period [15], whilst

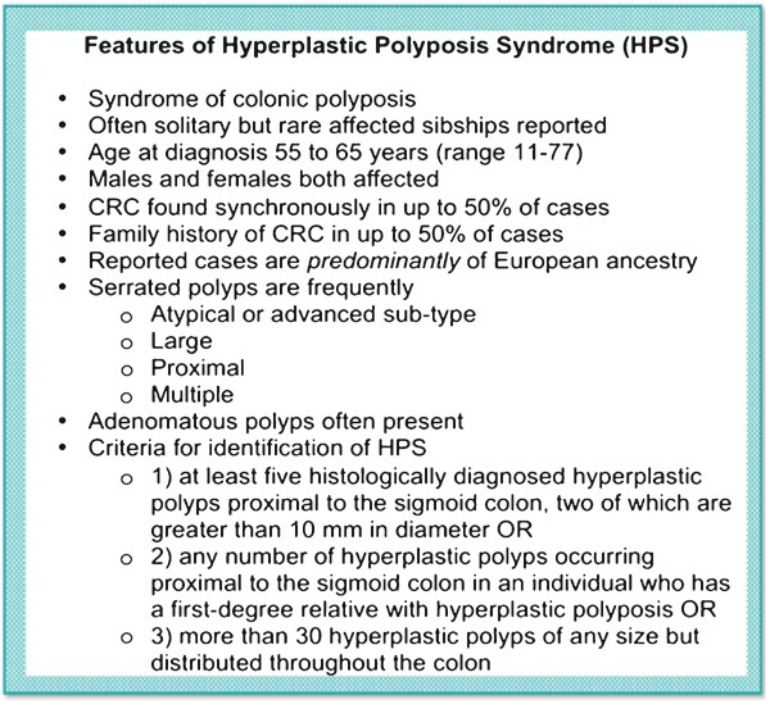

Fig. 6.1 Summary box: the features of HPS

Leggett and colleagues identified 12 cases from a similar institution during a 5-year period [23]. The age at presentation in HPS varies considerably among reported cases from 11 to 83 years [15], with most cases being diagnosed in their late 50s [18, 27] or early 60s [15]. However, in contrast to the sporadic setting, where advancing age is a factor for the development of serrated CRC [28], the clinical presentation of young-onset HPS resembles that seen in the older HPS patients, namely multiple polyps and a high incidence of synchronous CRC [14, 24, 26]. Though males are more numerous in several reports [15, 22], other published series provide no evidence for gender preponderance in HPS. Some large series suggest that a family history of CRC is a relatively frequent finding with figures of up to 59% [18, 19]. Studies of ethnicity in HPS are scant. Observations from Yeoman and colleagues demonstrated that, in a 24-case series from a large, multi-ethnic New Zealand patient group, all cases of HPS were derived from the European component, despite only 46% of the patient group having European ancestry [21]. At a population level, serrated pathway CRC is more frequent in individuals with northern European ancestry [29].

Though the most prominent phenotypic feature in HPS is the presence of numerous serrated polyps, ranging in number from 5 to greater than 150, and characteristically numbering from 40 to 100, a diverse range of lesions may be present, including atypical serrated polyps and serrated lesions with adenomatous features. It has been estimated that adenomatous lesions, including those with villous components and atypical serrated polyps, are seen in up to 90% of cases [15, 23]. By comparison, atypical serrated polyps are observed in only 4–11% of unselected patients undergoing colonoscopy [30, 31].

6.3 HPS and an Increased Risk of CRC: An Evolving Concept

The first detailed histological description of HPS was published in 1977 [16]. Among 801 epithelial polyps examined, this report made the observation that adenomatous foci could arise within serrated lesions. Subsequently, Cooke and colleagues described HPS as a variant of FAP [4]. A similar report describing cancer and dysplasia in a background of HPS was presented in 1979 [32]. Therein, Cooper and colleagues, whilst suggesting that hyperplastic polyps were ‘benign, non-neoplastic proliferations which unlike tubular and villous adenomas did not predispose the patient to colonic cancer’, went on to demonstrate a case of HPS where hyperplastic adenomatous transformation and cancer had probably occurred, and recognized that cancer could be associated with unusual cases of multiple hyperplastic polyps [5]. In 1980, seven cases of HPS were recorded from a London hospital [33]. Six of the seven cases were male and there was an average age at presentation of 37 years. Larger metaplastic polyps were frequent in these cases, and the possibility that ‘metaplastic polyposis’ was a pathological entity was raised [22]. However, despite follow-up, no cases of CRC were observed. Such disparities in the clinical consequences of HPS resulted in a slow recognition of the magnitude

of risk for malignancy in this condition although quantitation of risk still remains problematic. Many series and case reports have now been published [4–6, 12, 14–16, 18, 23–26, 34–54]. In the two largest series published to date, 26% of patients presented with at least one CRC [18, 27]. In an attempt to address the problem of malignant risk estimation, Rubio and colleagues conducted a pooled analysis from 30 publications and found that 67 of 137 (48.9%) cases of HPS presented with CRC [15], commensurate with one of the largest series published [18], and thereby confirming that HPS is a condition which carries a risk of CRC development well in excess of that seen in the general population, despite potential bias in patient ascertainment. A summary of the findings in the largest series of HPS cases is presented in Table 6.1.

6.4 Phenotypic Heterogeneity in HPS

Though polyposis is frequently pan-colonic, the CRC in HPS are significantly more likely to be located in the proximal colon [15, 21] and multiple malignant lesions may be present [6, 15]. Interestingly, the presence of carcinoma is more closely related to the type of co-existing polyps than to the absolute numbers of polyps present [15]. In reports of series where CRC was present, it appeared that the risk of CRC was higher in those with atypical or large serrated polyps (sessile serrated adenomas, serrated adenomas and mixed polyps, with adenomatous or villous changes [23, 43]). In a study of 10 cases from Sweden, the presence of a villous component was considered the most important harbinger of malignancy, with such lesions more likely to be observed in contiguity with an invasive carcinoma than classical serrated adenomas which, though significantly more numerous than expected, showed a malignant transformation rate equal to that seen in the sporadic setting [15]. Interestingly, the presence of atypical serrated polyps was more likely in cases where the absolute numbers of polyps were relatively low, suggesting a phenotypic dichotomy within HPS.

Genetic heterogeneity may underlie the variable phenotype seen in HPS. The issue of variable presentation in HPS was highlighted by Torlakovic and Snover [14] in 1996, and further analyzed by Rashid and colleagues in 2000. In the former report, six cases of HPS were described histologically, and the polyps were seen as more like serrated adenomas than those seen in classical cases of HPS. It was proposed that this particular sub-type should be noted as an indication of malignant risk. The lesions in question are now referred to as SSAs [55–59]. They occur also in the sporadic setting, and evidence suggests that they are precursor lesions which drive the serrated pathway of CRC development [59, 60].

The second report suggested that there are *at least* two sub-types of HPS. The first sub-type is characterized by numerous classical hyperplastic polyps which may or may not be large, and may have accounted for many of the cases in the series described by Williams et al. [33] and Ferrandez et al. [61]. An alternative sub-type demonstrates multiple lesions, though with lesser numbers than the first sub-type.

Table 6.1 Key findings in HPs from publications reporting more than five cases

Author	Year	Cases HPS (*n*)	Mean age (years)	% Males	Number polyps observed	% With CRC	CRC in proximal colon	Family history of CRC
Carvajal-Carmona [19]	2007	32	46	66%	11-Multiple	25%	NS	59%
Chow [18]	2006	38	44	55%	20-Multiple	26%	40%	50%
Renaut [27]	2001	28	58	54%	20-Multiple	29%	NS	39%
Yeoman [21]	2007	24	61	42%	5-Multiple	54%	84%	17%
Ferrandez [36]	2004	15	53	66%	15-Multiple	0%	NS	0%
Lage [43]	2004	14	58	NS	19–100	43%	67%	33%
Hyman [38]	2004	13	62	38%	20-Multiple	54%	71%	38%
Rashid [49]	2000	13	58	54%	Multiple	77%	NS	38%
Leggett [23]	2001	12	57	42%	30−>100	58%	NS	17%
Rubio [15]	2006	10	61	80%	6–159	70%	43%	10%
Spjut [16]	1977	9	53	NS	Multiple	11%	NS	NS
Williams [22]	1980	7	37	86%	50–150	0%	NS	14%
Torlakovic [14]	1996	6	57	83%	50–100	67%	NS	NS
Place [48]	1999	6	60	100%	50–100	50%	100%	14%
Averaged values			*53 years*	*58%*		*42%*	*67%*	*34%*

NS not specified or unknown

However, the lesions observed include a diversity of polyp sub-types including hyperplastic polyps, serrated adenomas, SSAs, traditional adenomas and polyps with mixed elements [27, 49]. This latter sub-type is more likely to have polyps with diameters exceeding 1 cm, dysplastic changes, to involve the proximal colon and to be associated with the presence of CRC [23]. That there are two forms of HPS has been suggested by others [62]. The reasons for this dichotomy are currently unknown. Possibilities include different rare germline mutations, or a common variant, which differentially interacts with genetic backgrounds or environmental modifiers.

6.5 Molecular Pathways in HPS

6.5.1 Historical Context

To understand the molecular pathways in HPS, it is first necessary to review the historical context within which the serrated pathway was first described, and how the threads of evidence have given rise to the model of the present day [63]. Traditionally, it had been thought that almost all CRCs developed within adenomatous polyps initiated by mutation of the tumour suppressor gene *APC* and subsequently transformed by inactivation of genes, including *TP53*. However, with the passage of time it was observed that only a portion of CRC had mutation of *APC* and *TP53* [64], raising the possibility of an alternative pathway. Such a pathway gained credence with the identification of the underlying genetic cause of Lynch syndrome in 1993 [65]. The phenotypic consequences of different stimuli or alternative genetic changes can be overlapping. The molecular phenotype of microsatellite instability (MSI) in CRC represents an example of this phenomenon. In Lynch syndrome, germline mutations in the DNA mismatch repair genes result in MSI due to the accumulation of unrepaired replication errors in repetitive DNA. MSI was *also* found to occur sporadically, and to account for 10–15% of all CRC [66]. In this sporadic form of DNA mismatch repair deficiency, an important alternative avenue of cancer progression through methylation silencing of tumour suppressor genes was subsequently identified, thus creating the foundation for the serrated pathway. Sporadic cancers with high-level MSI were referred to as MSI-H CRC, were more prevalent in the proximal colon and resulted from the epigenetic inactivation of the DNA mismatch repair gene *MLH1* [67].

While the evolution of the majority of CRC is consistent with the accepted adenoma–carcinoma model, the origin of sporadic MSI-H CRC was only explained in relatively recent times and amongst considerable controversy [37]. The serrated pathway was recognized at a molecular pathology level in 1999 by Jass and colleagues using mucin immunohistochemistry of CRC, in which MSI-H CRC and serrated polyps were shown to have overlapping mucin profiles [68, 69]. Further, the malignant transformation of advanced serrated polyps in a study of four cases of HPS published in the following year demonstrated that MSI-H

CRC arose due to the inactivation of *MLH1* in dysplastic foci within advanced serrated neoplasms [6]. All four MSI-H dysplastic foci and six MSI-H cancers in this report showed loss of MLH1 expression by immunohistochemistry. In addition, the apparent plasticity of the serrated pathway to produce CRC with MSI levels encompassing MSI-H as well as MSI-L (low-level MSI) and MSS (microsatellite stable) was also demonstrated in this seminal work. Specifically, a single case of HPS presented with six synchronous CRC, and all three levels of MSI were represented. Since commitment to an MSI-H phenotype occurs around the time of malignant transformation, this finding is unlikely to be due to the temporal context of sampling.

Description of the molecular genetic abnormalities present in serrated lesions has resulted in their being redefined as neoplasms. One of the critical events driving this change in approach has been the recognition of a serrated polyp-colorectal cancer developmental pathway associated with somatic oncogene mutation and gene promoter hypermethylation as an alternative to the adenoma–carcinoma sequence [70] characterized by chromosomal instability. In addition, observations that even within the earliest manifestation of serrated polyps, the aberrant crypt focus (ACF), activating mutations in the oncogenes *KRAS* and *BRAF* are detectable [71–75] have further consolidated the position of the serrated pathway as one of neoplastic change. Importantly, mutation profiles in the serrated pathway [76] are non-overlapping with those identified in the adenoma–carcinoma progression sequence [7] which is largely characterized by chromosomal instability.

6.5.2 Serrated Pathway Changes in HPS

The somatic molecular features of HPS lesions are consistent with those identified in their sporadic counterparts, particularly, activating mutations in *BRAF* [77–79], and widespread hypermethylation of gene promoters [80] with or without microsatellite instability (MSI) [37, 81]. In addition, these serrated pathway features demonstrate a high rate of concordance within individual lesions in those with HPS [77, 80]. Further, increased methylation of gene promoters is evident even in the normal mucosa of individuals with HPS [80, 82, 83], indicating that an epigenetic regulatory defect may be present in the normal tissues of individuals with HPS. Though MSI-H *BRAF* mutation-bearing CRC can occur in HPS [6], and in CRC families with serrated neoplasia [84], CRC in HPS are more likely to be non-MSI-H [18]. Importantly, in the North American population, non-MSI-H *BRAF* mutation-bearing CRC demonstrate the strongest association with a family history of CRC [85]. The association of female gender with CIMP cancers is largely confined to the MSI-H subset [86] and to individuals of an advanced age [28]. There is neither female predominance, nor a majority of MSI-H CRC in HPS, suggesting that late-onset serrated CRC arises via an alternative mechanism.

6.5.3 Molecular Heterogeneity in HPS

The question of heterogeneity in HPS can be further considered with an examination of the reported molecular changes in the syndrome. Mutations in the oncogene *KRAS* are found in small distal hyperplastic polyps [76, 87], and it is known that the presence of *BRAF* and *KRAS* mutations are mutually exclusive in neoplastic tissue [88]. In a previous study, Rashid and colleagues classified HPS cases as follows: (1) 13 subjects (including three from one family) with hyperplastic polyposis (>20 hyperplastic polyps), (2) five subjects with less than 20 hyperplastic polyps (1–14) but at least one polyp in excess of 1 cm in diameter and (3) five subjects with multiple, small hyperplastic polyps but less than 20 in number [89]. This study did not identify SSAs nor were polyps typed according to *BRAF* mutation status due to its year of publication. However, an important finding from this study was that *KRAS* mutation was commonly observed in hyperplastic polyps from subjects with multiple lesions, but was not found in hyperplastic polyps from cases where large hyperplastic polyps were seen. It is now known that such patients are more likely to demonstrate somatic *BRAF* mutation [77], as opposed to the *KRAS* mutations seen in small typical hyperplastic polyps, particularly in the distal colon [49, 76]. Since the report by Rashid et al. [89] was published it has become clear that the most frequent genetic alterations in large hyperplastic polyps or SSAs include mutation of *BRAF* and CIMP [90–93]. However, rare cases of HPS are reported where *KRAS* mutations predominate, and current evidence suggests that in at least some of these patients, germline mutation of *MUTYH* may be responsible [18, 19, 83, 94].

Although the possibility of these two types of HPS was first raised over 10 years ago [62], the application of such a classification to CRC risk may not be readily implemented as even though large and dysplastic lesions are more likely to be an indicator of high malignant potential, the presence of CRC in cases with multiple small hyperplastic polyps [27] as well as the lack of CRC in some cases of HPS with large and atypical lesions argues against a non-overlapping classification.

6.6 Serrated Pathway Cancers in the Population

As previously stated, a significant proportion of population CRC develops within SSAs, a feature shared with individuals who have HPS. Because the molecular profiles of HPS tumours and those in the population developing via the serrated pathway are largely overlapping at a fundamental level, this suggests a common genetic aetiology [10]. In particular, cancers with CIMP and *BRAF* mutation arise in a sub-type of serrated polyps called SSA [55]. Spring and colleagues [31] found that the presence of at least one SSA was associated with increased polyp burden consistent with an underlying predisposition. In addition, an association between family history of CRC and advanced serrated polyps was observed. SSAs are identified

amongst lesions removed at colonoscopy with ranges from 2 to 9% [30, 31]. In keeping with their status as a precursor lesion for CIMP CRC, SSAs demonstrate a high level of *BRAF* mutation [78], CIMP and a proximal predilection [31].

The population characteristics of CRCs featuring somatic *BRAF* mutation and CIMP have been extensively studied, and indications are that they represent a distinct entity associated with their own genetic and environmental risk factors [95–97]. In particular, a large population-based cohort of over 800 cases from North America [96] has been utilized to study the epidemiology of CIMP CRC. The investigators unequivocally demonstrated CIMP within this population [95], occurring in 30% of CRCs. In a subsequent study of this population group, CIMP microsatellite stable tumours showed a trend associated with a positive family history of CRC. However, when cases from the same population were analysed for somatic *BRAF* mutation, family history of CRC was significantly associated with *BRAF* mutation positive microsatellite stable cancers (OR 4.2; 95% CI 1.65–10.84). This result raised the possibility of a genetic predisposition to develop CRC with *BRAF* mutation [98], that is, to develop CRC with the molecular genetic hallmarks of HPS.

6.7 Genetics of HPS

Though multiple cases of HPS are rarely reported within kindreds, a family history of CRC in patients with HPS has been reported by several investigators [40, 43, 49], and occurs relatively frequently. Descriptions of HPS in a family setting were reported from New Zealand in 1996 and in 1997 [39, 40]. A family with an affected mother and five of her offspring developing CRC was described by Jeevaratnum and colleagues [40]. This family demonstrated multiple, large, hyperplastic polyps as well as low-level MSI in a proportion of their CRC, but lacked the features of either FAP or Lynch syndrome. Rashid et al [49] described three kindreds in which there were 11 individuals with HPS, and three of these (27%) also had CRC. Chow and colleagues carried out extensive studies of the genetic etiology of HPS in a series of 38 HPS cases. In this study, 19 (50%) of HPS patients reported a first-degree relative with CRC, and two had a family history of HPS [99], including a consanguineous family. A Portuguese study revealed a family history of CRC or polyps in 6 of 12 (50%) patients with HPS [43]. Azimuddin and colleagues described 16 cases of large atypical hyperplastic polyps from a series of colonoscopies [34]. The lesions were concentrated in the proximal colon, and 9 of 16 cases had a family history of CRC, with an increased likelihood where the polyps demonstrated dysplastic changes [34]. The presence of a family history of CRC in HPS was relatively low in some published series [23], despite a high personal history of CRC, and the reasons for these observations are currently unclear.

Taken together, the preceding data suggest that HPS is likely to represent a novel syndrome of CRC predisposition with a risk to relatives which exceeds that of the population. The nature of such a predisposition remains speculative at present, however, the phenotype of multiple neoplasms, and occasional affected sibships

including consanguineous kindreds [18] suggest a pattern of inheritance consistent with an autosomal recessive or co-dominant mode. Co-dominant modes of inheritance result in an intermediate phenotype when one variant risk allele is present, and a significantly altered phenotype in those where both alleles are variants. Our current knowledge of the prevalence of HPS in the population does not allow us to calculate with any accuracy the proportion of carriers of a single hypothetical risk allele, nor does such an approach allow for the presence of genetic heterogeneity. However, given that up to 50% of HPS individuals report a family history of CRC [18, 23], it is possible that the burden of serrated pathway CRC in the population may be accounted for by a portion of carriers of a single risk allele of HPS (Fig. 6.2). Such individuals may develop a small number of serrated polyps [10] and a subset of these may evolve into a serrated pathway CRC (Fig. 6.3). This model is consistent with CRC causation by common less penetrant co-dominant alleles [8]. There is increasing support in the literature for common lower penetrance cancer susceptibility alleles present at increased frequency in CRC cases with strong family histories compared to consecutive case series [100]. Such a mechanism has been proposed for another recessive polyposis syndrome, *MUTYH*-associated polyposis (MAP) [101–109]. The magnitude of residual familial risk is, however, currently unknown. The identification of the genetic variant associated with HPS will be a necessary first step in the examination of the model proposed in this chapter. Sequence variants in *MYH* and *EPHB2* have been reported in rare HPS cases though these did not account for the majority of cases seen in the respective studies [18, 110, 111].

HPS has many hallmarks of a genetic predisposition (Fig. 6.4). These include an earlier age of onset when compared with sporadic cases of CRC [28]. The mean age of diagnosis is usually between 55 and 65 years; however, HPS with CRC has been reported from both an 11-year-old girl and a man aged 24 years [112, 113]. The number of polyps that develop in HPS typically ranges from 40 to 100 and suggests that a pre-neoplastic field exists in the colon, consistent with a germline genetic change. This is supported by the finding of extensive methylation in the normal mucosa of HPS cases [114]. Though environmental factors could be involved, the consistent phenotype of the proposed response also indicates a germline predisposition. Not only are there multiple polyps in HPS, but the risk of multiple cancers is greater than in the general population. Such cancers may number up to six in a single case patient [115], and highlights the malignant potential of the polyps in HPS.

Other indicators of a familial predisposition include the presence of polyps and cancers in the first-degree relatives of subjects with HPS [116]. Conversely, examples of HPS have been described in families meeting the Amsterdam criteria but lacking germline defects in a DNA mismatch repair gene [117]. Such familial cancer syndromes associated with *BRAF* mutation-bearing tumours have been described from Sweden [98] and Australia (where 2 of 11 CRC families included cases of HPS) [84]. CRC occurring across the members of these families show the molecular features of colorectal cancers occurring in patients with HPS: variable MSI status, somatic *BRAF* mutation and DNA methylation [117]. It is currently not known whether these families constitute a part of the HPS spectrum, carrying one putative co-dominant allele or whether they represent a separate syndrome.

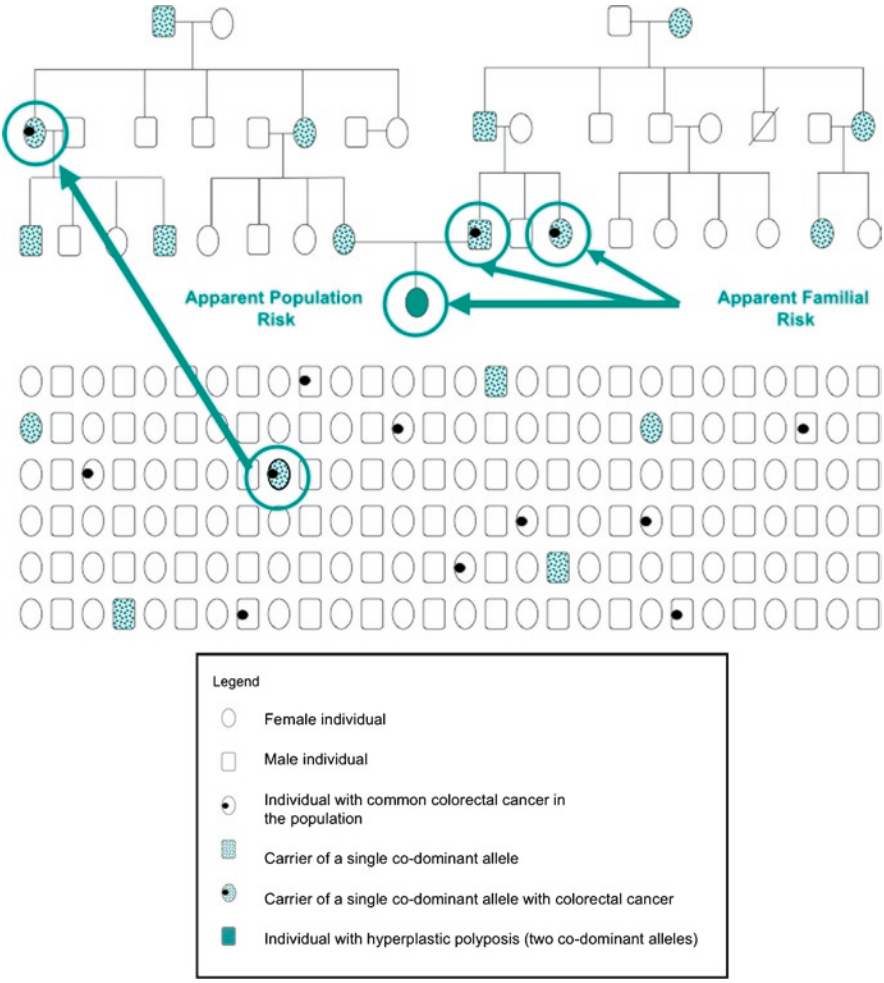

Fig. 6.2 Schematic of serrated pathway CRC in families and in the population. *Adapted from Young et al., 2007* [124]. A hypothetical family is depicted in the upper panel of the diagram. Co-dominantly inherited HPS (individual with solid symbol) assumes that both parents carry a single co-dominant allele and that one of their parents in turn also carries a single co-dominant allele. A subset of the carriers in a family may develop CRC, as has been reported previously [18, 38, 43]. Carriers of one co-dominant allele are shown as mottled symbols. A simulated segment of the population is depicted in the lower panel. Carriers of one co-dominant allele, most of whom are likely to be asymptomatic, would be distributed throughout the population. A subset of the population will develop CRC, and less than one in 10 of these cases will be non-MSI-H serrated pathway CRC. Some individuals are more likely to be identified as a family at risk due to the number of cases with colorectal neoplasms. However, a single carrier in the family with CRC would appear as an isolated case of serrated pathway CRC in the population indistinguishable from the population-based CRC which is characterized by *BRAF* mutation and CIMP

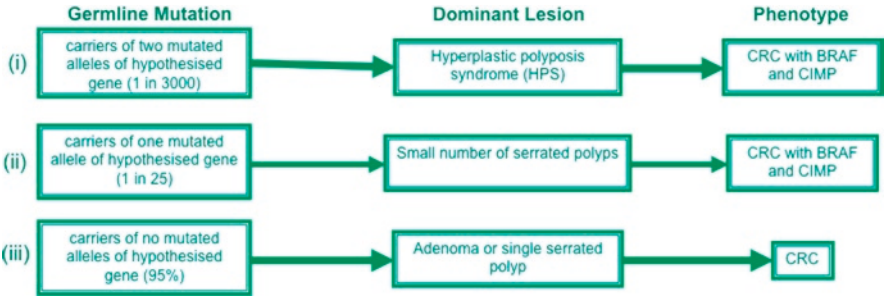

Fig. 6.3 Model for the distribution of alleles and phenotypes in the population. *Adapted from Young et al., 2007* [124]. Diagram demonstrates the phenotypic consequences of (i) Two putative co-dominant HPS alleles, (ii) One putative co-dominant HPS allele and (iii) Non-carrier

HPS: Evidence for Genetic Predisposition

- HPS cases develop multiple serrated lesions
- The normal tissue in HPS shows pre-neoplastic change
- HPS has an earlier age of onset for CRC than is seen in the general population
- HPS predisposes to CRC, and is more likely to be multiple than in the general population
- Multiple cases of HPS have been reported in rare families
- A family history of CRC and polyps has been reported in up to 50% of cases of HPS
- Association with European ethnicity within a single multi-ethnic population
- The presence of advanced serrated polyps in the population is more likely to be associated with increased polyp burden and a positive family history of CRC
- Population-based individuals with somatic *BRAF* mutation in their cancers are more likely to have a family history of colonic neoplasia than those with common colorectal cancer

Fig. 6.4 Summary box: HPS as a genetic predisposition to CRC

Most reports of HPS arise from European populations, including approximately 40% from Australasia. Reports of HPS from Asian populations include only rare case reports from Japan, and such a striking difference between Europeans and Asians particularly where there is shared environmental risk [21] supports a particular type of germline predisposition consistent with an ancestral haplotype. Interestingly, FAP and Lynch syndrome are genetic predispositions to CRC, which are reported from diverse ethnic groups.

6.7.1 A General Cancer Predisposition?

Recently, intriguing findings have emerged from a study by Vandrovcova and colleagues using a cohort of non-FAP, non-Lynch syndrome cases from Sweden [98]. Interestingly, families with extra-colonic tumours showed a much higher mutation frequency of somatic *BRAF* mutation (17.5%) compared with families with colonic cancer only (3.5%; $P = 0.009$). This striking dichotomy was further emphasized when it was demonstrated that 448 family members in *BRAF*-associated kindreds had significantly less adenomas compared with those from families with no *BRAF* mutation (odds ratio 8.5; CI 1.1–64.6). These findings demonstrate evidence for a germline predisposition to develop CRC with *BRAF* mutation and suggest that the underlying germline mutation, even though it has its greatest effect in the colon, confers a cancer predisposition which affects other organs including breast, pancreas, stomach, brain, lung, cervix, prostate, blood and skin.

6.8 Clinical Implications of HPS

CRC is a cause of significant cancer-associated mortality and morbidity in Western populations. The implications of a genetic predisposition to serrated neoplasia are considerable [10]. The two most important aspects of risk in HPS patients are those of CRC in the individual with this condition, and in his relatives. Colorectal cancers with *BRAF* mutation develop in a subset of serrated polyps called SSAs [55]. Given the prevalence of HPS in Europeans, it is likely that SSAs may also be more common in the wider European population. Several reports have suggested that malignant transformation in the serrated pathway may be unusually rapid in some clinical settings. Hyman and colleagues reported three cases of HPS where CRC developed despite two-yearly colonoscopy [38]. Similarly, Azimuddin and colleagues reported that three-yearly colonoscopy was inadequate for some families with atypical serrated polyps [34]. In addition to these case reports, more extensive studies have been conducted which have focused on interval cancers. Lazarus and colleagues suggested that serrated neoplasms are more likely to account for the occurrence of interval cancers [118]. Interval cancers have been found to be three times more likely to occur in the proximal colon [119], and almost four times more likely to be MSI-H [120]. However, the apparent rapid evolution to cancer of advanced serrated polyps remains enigmatic, and may be due to the difficulty of visualizing flat serrated lesions at colonoscopy. Currently these issues are unresolved, and recommendations for frequency and modality of CRC screening in individuals with HPS and their families, remain undefined [121].

6.9 Summary and Conclusion

Without a known germline sequence variant, the identification and management of individuals and their families with a CRC predisposition syndrome becomes increasingly problematical. The prospect of a syndrome with a co-dominant mode of inheritance presents particular difficulties in that although some individuals will present with a florid phenotype, such as that seen in HPS, first-degree relatives may have only a few polyps or none at all, as such disorders lack a defined phenotypic perimeter. The role of genetics departments in assembling the clinical picture families such as these is likely to become increasingly important. A more complete assessment including pedigree analysis, as well as pathology review and molecular and immunohistochemical phenotyping on multiple cases, may be of benefit to the diagnosis and management of some families, as confusion can be generated when diagnosis of a family is based on the assessment of single individual. This is especially true in families with serrated neoplasia where the presence of MSI or immunohistochemical absence of *MLH1*, coupled with an Amsterdam-like pedigree structure can erroneously lead to a diagnosis of Lynch syndrome [84]. Families segregating several germline variants have been reported [122], and the presence of a common low penetrance variant for HPS could contribute to a confusing presentation [123].

The concept that CRC can arise in advanced serrated polyps is now widely accepted. In addition, it is likely that the development of advanced serrated polyps may be associated with a common genetic predisposition. The existence of families and individuals with HPS, the increased prevalence of HPS in Europeans and the significantly increased risk for a family history of CRC in population-based cases of *BRAF* mutation-bearing CRC all suggest a genetic predisposition to develop advanced serrated lesions. Cases with HPS may represent the most clinically apparent manifestation of a widespread predisposition in the population.

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Chapter 7
MUTYH-Associated Polyposis

Jeremy P. Cheadle

Abstract MUTYH-associated polyposis (MAP) is an autosomal recessive disorder characterised by multiple colorectal adenomas and carcinoma. It is caused by inherited mutations in the human MutY homologue gene (*MUTYH*). MUTYH functions as a base excision repair DNA glycosylase that excises adenines misincorporated opposite 8-oxo-7,8-dihydro-2'-deoxyguanosine, one of the most stable products of oxidative DNA damage. The failure to correct this mispair is thought to cause the characteristic signature of G:C to T:A mutations found in MAP-associated tumours.

Keywords MUTYH • MYH • MAP • Colorectal cancer • APC • FAP

7.1 Inherited Predisposition to Colorectal Cancer

Inherited factors are thought to play a significant role in up to one third of colorectal cancers (CRCs), but only a minority of these can be accounted for by established CRC predisposition genes [1]. Familial adenomatous polyposis (FAP) (MIM 175100) is an autosomal dominant disorder characterised by the development of hundreds or thousands of colorectal adenomas (CRAs), some of which progress to cancer. Patients with FAP often have extracolonic manifestations including congenital hypertrophy of the retinal pigment epithelium, upper gastrointestinal tumours, desmoid tumours, hepatoblastoma, epidermoid skin cysts and benign osteoid tumours (Gardner's Syndrome) and cerebellar medulloblastoma (Turcot syndrome). FAP is caused by inherited mutations in the adenomatous polyposis coli (*APC*) gene that acts as a gatekeeper regulating the proliferation of colonic cells [2]. Tumours develop in patients with FAP after somatic inactivation of the wild-type *APC* allele in accordance with

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Knudson's '2-hit hypothesis'. Attenuated FAP (AFAP) is associated with smaller numbers of adenomas and is caused by germline mutations in the extreme 5′ or 3′ ends of *APC*, or in the alternatively spliced region of exon 9 [2]. Tumour development in at least some cases of AFAP appears to require somatic second and third hits of the wild-type and attenuated *APC* alleles. Hereditary non-polyposis CRC (HNPCC; MIM 114500) is an autosomal dominant disorder characterised by early-onset CRC (in the absence of florid polyposis) and other extra-colonic cancers, notably endometrial cancer and cancers of the stomach, small bowel, ureter and renal pelvis. HNPCC is caused by inherited deficiencies in the mismatch repair (MMR) pathway [3]. Germline mutations are most frequently found in *MSH2* and *MLH1*, and cause a high degree of somatic microsatellite instability (MSI) in the associated colorectal tumours. Mutations in *MSH6* are less frequent and are associated with less marked MSI. Tumour development in HNPCC requires somatic inactivation of the wild-type MMR allele, again in accordance with Knudson's '2-hit' hypothesis [3].

7.1.1 Identification of an Unusual Mutator Phenotype in Family N

In 2002, Al-Tassan and colleagues studied a British family (Family N) with three affected siblings with multiple CRAs and carcinoma [4]. Sequencing of the entire *APC* open reading frame (ORF) in germline DNA samples from two of the affected siblings, together with haplotype and expression analyses, excluded an inherited *APC* gene defect [4]. Assessment for MSI in DNA extracted from 11 tumours from Family N, also excluded a defect in MMR. To provide a clue as to the underlying genetic defect, the investigators sequenced the *APC* ORF in each of the 11 tumours and identified 18 somatic *APC* mutations, 15 of which were G:C→T:A transversions. This class of mutations accounts for only some 10% of reported somatic *APC* mutations, with frameshift mutations and loss of heterozygosity being the more usual classes of mutations leading to somatic inactivation of *APC* in colorectal tumours [4, 5]. Comparison of the findings in Family N with a database of somatic *APC* mutations from sporadic and FAP-associated colorectal tumours, confirmed that the excess of G:C→T:A transversions in Family N ('the mutator phenotype') was highly significant ($P = 10^{-12}$).

7.1.2 Base Excision Repair

The Base Excision Repair (BER) pathway plays a significant role in the repair of mutations caused by reactive oxygen species (ROS) that are generated during aerobic metabolism [6]. BER also protects against damage to DNA from methylation, deamination, hydroxylation and other by-products of cellular metabolism. BER is a multi-step process that involves the sequential activity of several proteins. DNA glycosylases initiate this repair pathway by recognising and removing a damaged or improper base by hydrolysing the N-glycosidic bond. At least ten DNA

glycosylases have been characterised and cloned in humans, and each excises an overlapping subset of oxidised, deaminated, alkylated, or mismatched bases [7]. To complete the repair process, the apurinic/apyrimidinic site is further processed by an incision step, DNA synthesis, an excision step, and DNA ligation through either the short or long-patch BER pathways. Although inherited deficiencies involving components of the nucleotide excision repair, MMR and recombinational repair pathways had all been linked to specific human genetic disorders, as of early 2002, no inherited disorder of BER had been identified [8].

8-oxo-7,8-dihydro2'deoxyguanosine (8-oxoG) is the most stable product of oxidative DNA damage [9] and readily mispairs with adenines [10], leading to G:C→T:A mutations in repair-deficient bacteria and yeast [11–13]. In *Escherichia coli*, three enzymes help protect cells against the mutagenic effects of guanine oxidation [12]. The BER DNA glycosylase MutM removes the oxidised base from 8-oxoG:C base pairs in duplex DNA, the BER DNA glycosylase MutY excises adenines misincorporated opposite unrepaired 8-oxoG during replication, and the 8-oxo-dGTPase MutT prevents the incorporation of 8-oxo-dGMP into nascent DNA (Fig. 7.1). Homologues of *mutM*, *mutY* and *mutT* have been identified in human cells and termed *OGG1* [14], *MUTYH* (*MYH*) [15] and *MTH1* [16], respectively.

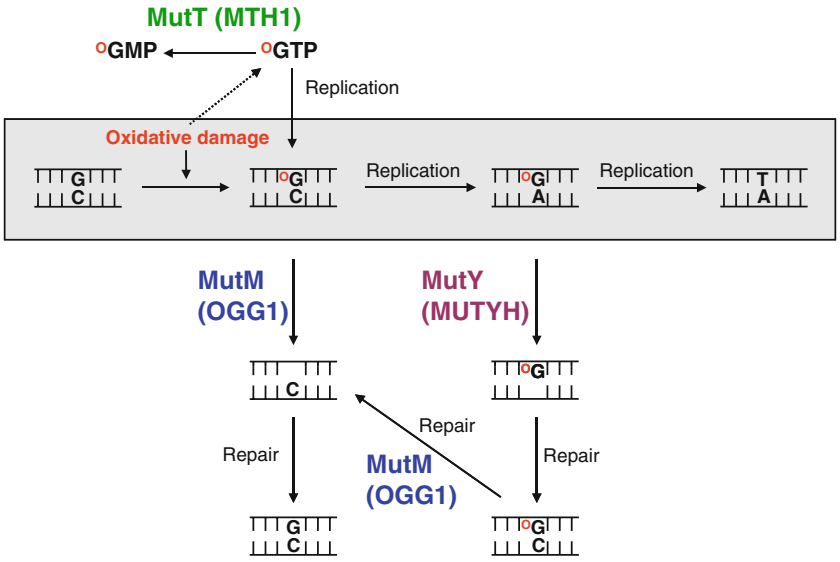

Fig. 7.1 The 8-oxoG repair system in *Escherichia coli*. MutT (human orthologue MTH1), an 8-oxo-dGTPase, prevents the incorporation of 8-oxo-dGMP into nascent DNA, MutM (human orthologue OGG1) DNA glycosylase removes the oxidised base from 8-oxoG:C base pairs in duplex DNA, and MutY (human orthologue MUTYH) DNA glycosylase excises A residues misincorporated opposite unrepaired 8-oxoG during replication. 8-oxoG readily mispairs with A residues, leading to G:C→T:A mutations in *MutM* and *MutY*-deficient bacteria (*grey box*). 8-oxoG is denoted by ^{o}**G**

7.1.3 Inherited Mutations in MUTYH Predispose to Colorectal Tumours

To determine whether an inherited defect in the 8-oxoG repair pathway was responsible for the pattern of somatic G:C→T:A mutations in Family N, Al-Tassan et al. [4] sequenced the ORFs of *OGG1*, *MUTYH* and *MTH1* in a blood DNA sample from an affected sibling. Two non-conservative amino acid variants were identified in *MUTYH* (Y165C and G382D), but no likely pathogenic changes were identified in *OGG1* or *MTH1*. All three affected siblings from Family N were found to be compound heterozygotes for Y165C and G382D and the unaffected family members were either heterozygous for one of these variants or normal, suggesting transmission as an autosomal recessive trait (Fig. 7.2). Consistent with this, no somatic mutations in *MUTYH* were identified upon comprehensive analysis of the 11 colorectal tumours from Family N [4].

In an attempt to identify further cases, Jones et al. [17] sequenced the *MUTYH* ORF in 21 unrelated patients with multiple CRAs with or without carcinoma, and

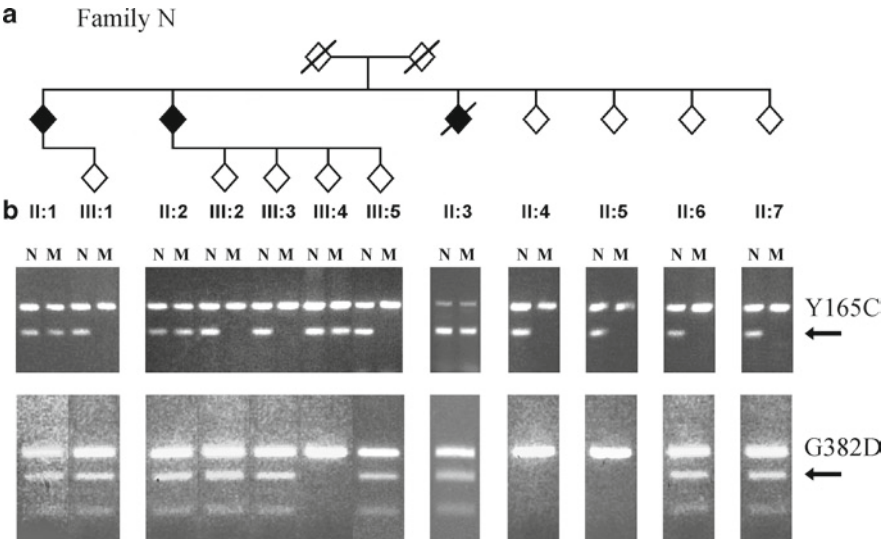

Fig. 7.2 Segregation of germline *MUTYH* variants in Family N. (**a**) Pedigree of Family N: II:1 and II:2 were found to have adenomas at 50 and 46 years of age. Both had approximately 50 macroscopically visible adenomas at colectomy at 59 and 55 years of age. II:3 died following discovery of a colonic adenocarcinoma and an adjacent adenoma at 46 years of age, but without full assessment of the large bowel. II:4–7 and III:1–5 were normal on colonoscopic assessment. (**b**) Screening for Y165C (an A to G substitution at nucleotide 494) by amplification refractory mutation system (ARMS) and G382D (a G to A substitution at nucleotide 1145) by a *Bgl*II digest revealed that the three affected siblings (*filled symbols*) were compound heterozygotes for these *MUTYH* missense variants, while normal family members (*non-filled symbols*) were either heterozygous for one of the variants or normal. *N* normal ARMS reaction, *M* mutant ARMS reaction. *Arrows* indicate the positions of the mutant alleles

identified seven patients with biallelic germline *MUTYH* mutations including four cases homozygous for nonsense changes. The absence of any history of CRAs or carcinoma in the obligate heterozygote parents and the occurrence of adenomatous polyposis in two siblings of one index case, was consistent with the transmission as an autosomal recessive trait [17]. These findings confirmed that biallelic inherited mutations in *MUTYH* predispose to multiple CRAs and CRC, and showed (for the first time) that inherited mutations in the BER pathway had a major phenotypic consequence. Analysis of somatic *APC* mutations in CRAs and carcinomas from these seven patients again revealed a highly significant excess of somatic G:C→T:A mutations, as compared to sporadic or FAP-associated colorectal tumours, confirming the mutational basis underlying this disorder [17]. This disorder has been termed *MUTYH*-associated polyposis (MAP).

7.2 The Phenotype of MAP

Mutation analysis of *MUTYH* has now been undertaken in several series of patients with FAP-like and AFAP-like phenotypes and in whom no inherited *APC* mutation could be identified [17–23]. Biallelic *MUTYH* mutations have been identified in approximately 25% of such cases and, in general, segregation has been consistent with transmission of MAP as an autosomal recessive trait with high and probably complete penetrance. The colorectal phenotype of MAP closely resembles AFAP (10–100 adenomas), or 'moderate' FAP (100–1,000 adenomas), but not severe FAP (>1,000 adenomas). We have proposed that this may reflect the number of somatic mutations required for initiation of adenoma development [24]. In FAP, adenoma development requires only a single somatic *APC* mutation. Families with biallelic *MUTYH* mutations may be more comparable to patients with AFAP who develop smaller numbers of adenomas that require two somatic *APC* mutations for initiation. By contrast, most patients with HNPCC develop only one or a few adenomas or carcinomas whose initiation requires somatic inactivation of a wild type MMR allele and two somatic *APC* mutations in the target cell.

It is important to note that some cases with biallelic *MUTYH* mutations appear to develop fewer than ten macroscopic adenomas by middle age and to have developed CRC in the absence of obvious polyposis [18, 25, 26]. As expected for a recessive trait, many cases appear to be sporadic and hence present symptomatically and CRC was found at presentation in ~50% of cases reported by Sampson et al. [18] and by Seiber et al. [19]. Duodenal adenomas have been reported in some patients and clinical studies of further patients are required to establish whether other extra-colonic manifestations also occur at significant frequencies.

A possible explanation for the predominantly colorectal phenotype in MAP is the high level of oxidative damage that occurs in the large bowel [27]. An alternative or additional factor was proposed after careful examination of the target

sequence surrounding the somatic G:C→T:A mutations in MAP tumours – the two bases immediately 3′ to the mutated G are almost always AA and this preponderance of G:C→T:A mutations at GAA sequences is highly significant [4, 17]. Interestingly, this sequence specificity occurs irrespective of the nature of the germline *MUTYH* mutations [17]. *APC*, the key gatekeeper in colorectal tumourigenesis, has a total of 216 GAA sites in which G:C→T:A mutations could lead to termination codons. By comparison TP53, PTCH, RB1 and VHL (that are frequently mutated during tumourigenesis in the brain/breast/lung, skin, retina and kidney) have significantly fewer target sites and therefore *APC* may be a particularly vulnerable target for mutagenesis in MAP [24].

7.2.1 Mutation Spectrum in MUTYH and Diagnostic Implications

As of May 2007, 31 mutations that are predicted to truncate the protein product have been reported in *MUTYH*, comprising 11 nonsense, ten small insertion/deletions and ten splice site variants (Fig. 7.3). In addition, 53 missense variants and three small inframe insertion/deletions have been reported that are distributed throughout the gene ([4, 17–23, 28–49, reviewed in 50]). Although there is some reporting bias, the missense variants Y165C and G382D together account for approximately 73% of all *MUTYH* mutations reported to-date, and have been identified commonly in the British, Italian, American, Portuguese and Dutch populations (reviewed in [51]). In addition, specific mutations in *MUTYH* have been identified in different populations and diagnostic screening strategies will have to be optimised accordingly. For example, recurrent mutations have been identified in Italian (1395delGGA), Portuguese (1186-1187insGG) and Dutch patients (P391L) and the truncating mutation E466X has been identified in at least four unrelated Gujarati families [18, 20, 22, 38]. Apart from Y165C and G382D, most missense variants are rare; however, their collective frequency and the lack of functional data for the vast majority pose major difficulties for molecular diagnostics since many will be benign polymorphisms. Most of these variants remain ‘of uncertain pathogenicity’ and genetic counselling for patients carrying them is problematic.

7.3 The Pathway of MAP Tumourigenesis

CRCs appear to develop according to particular genetic pathways. The most common pathway is characterised by mutations of the *APC* and *p53* genes, by 18q allelic loss, by mutation of *K-ras* and *SMAD4* in some cases, and by an aneuploid/polyploid karyotype; these tumours are said to have followed the chromosomal instability (CIN) pathway. Alternatively, ~15% of sporadic CRCs show

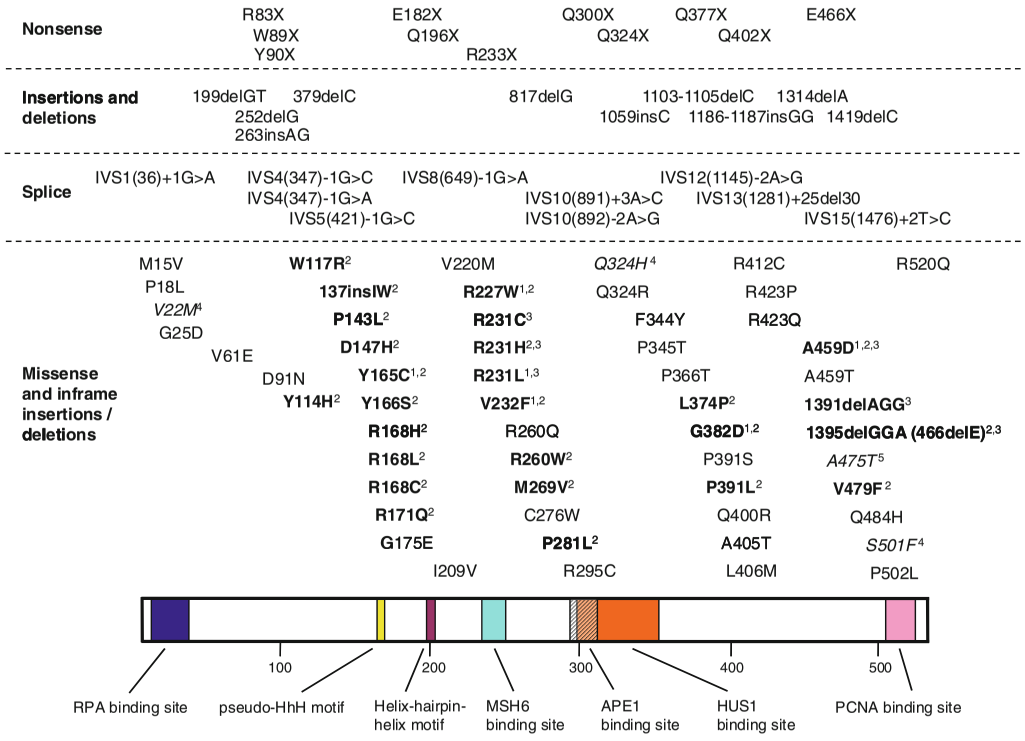

Fig. 7.3 Spectrum and distribution of truncating mutations, missense and inframe insertion/deletion variants identified in *MUTYH* (as of May 2007). Approximate positions of putative functional domains are indicated in relation to the *MUTYH* coding region. Missense variants proven or likely to be pathogenic are highlighted in *bold* ([1]data demonstrating functionally compromised, [2]rare variant found in combination with a proven *MUTYH* mutation in a patient with CRAs, [3]rare variant found in a homozygous state in a patient with CRAs), those that are unlikely to be pathogenic are *italicised* ([4]common polymorphism, [5]identified in cases with biallelic *MUTYH* mutations) and those that are undefined are in *regular font*

MSI (primarily due to hypermethylation of the *hMLH1* promoter), aberrant DNA mismatch repair, a near-diploid karyotype, and lower levels of *p53*, *SMAD4*, and *K-ras* mutation, but higher frequencies of *BAX*, *TGFBIIR* and *BRAF* mutation. Yet additional CRCs have neither MSI nor an aneuploid/polyploid karyotype and are termed MSI- CIN-.

Consistent with the G:C→T:A mutator phenotype observed in *APC*, a proportion of MAP adenomas show a specific, activating missense mutation of *K-ras* (G12C) that also results from a G:C→T:A transversion [52, 53] As expected, MSI is not a feature of MAP tumours [4]. The role of CIN in MAP-associated tumours is unclear; some investigators have reported that MAP tumours appear to be near diploid whereas others have shown that up to 80% of MAP polyps are aneuploid [53, 54].

7.4 Function of MutY/MUTYH

The MUTYH protein has been difficult to express and only limited biochemical data is available. Parker et al. [55] have shown that it interacts with AP endonuclease, PCNA and RPA, suggesting a role in long patch BER, and Boldogh et al. [56] have shown an association with the replication foci, suggesting a role in replication-coupled repair. More recently, Shi et al. [57] have shown that MUTYH interacts with hHus1 (human Hus1) and hRad1 (human Rad1) which are components of the 9-1-1 (Rad9, Rad1 and Hus1) DNA damage sensor complex. The major Hus1-binding site was localised to residues 295–350 of MUTYH and the interaction was enhanced following ionising radiation.

More extensive structural and biochemical information is available on MutY. The N-terminal domain of MutY contains the catalytic region [58] and shares several motifs with other BER glycosylases, including the helix-hairpin-helix (HhH), pseudo HhH and the iron–sulphur cluster loop motif [59]. MutY contains a C-terminal domain that is not found in the BER glycosylase superfamily, with sequence and structural homology to MutT (an 8-oxoGTPase) [60] and the C-terminal domain of MUTYH correspondingly shares homology with the human orthologue of MutT (MTH1) [61]. NMR and biochemical studies have suggested that the C-terminal domain plays a role in 8-oxoG recognition [60, 62, 63]. Like all DNA-nucleotide-modifying enzymes, MutY has to recognise and access chemical adducts on DNA bases hidden within the double helix of DNA. These enzymes expose their targets by rotating the phosphodiester bonds surrounding the nucleotide, causing the target base to be flipped out of the DNA helix. Crystallographic studies on *Bacillus stearothermophilus* MutY interacting with DNA containing an 8-oxoG:A mispair [64] show that MutY residues deeply penetrate the DNA helix, interrupting helical stacking on both strands causing a sharp bend and extrahelical extrusion of the substrate adenine.

7.4.1 Functional Overlap with Other Repair Pathways

The MMR system serves to increase the fidelity of DNA replication and genetic recombination and the proteins involved also have roles in transcription coupled repair (TCR), meiosis, cell cycle arrest and apoptosis. For mismatch recognition, the MSH2 protein forms a heterodimer with MSH6 or MSH3 depending on whether base–base mispairs (MSH2/MSH6) or insertion–deletion loops (MSH2/MSH3 and/or MSH2/MSH6) are repaired [3]. MUTYH interacts with the heterodimer MSH2/MSH6 via MSH6, and MSH2/MSH6 stimulates the DNA binding and glycosylase activities of MUTYH with an 8-oxoG:A mismatch [65]. Because both MUTYH and MSH6 interact with PCNA and co-localise to the replication foci, PCNA may act as a co-ordinator of both repair pathways. Therefore, MUTYH-mediated BER may co-operate with MMR in protecting against the mutagenic effects of 8-oxoG. Other repair pathways have also been implicated in the repair of 8-oxoG; the Cockayne syndrome B gene product may be required for general genome repair [66], and BRCA1 and BRCA2 required for TCR [67].

7.4.2 Functional Studies of *MUTYH* Missense Variants

To-date, functional studies have been performed on just six *MUTYH* missense variants (Y165C, R227W, R231L, V232F, G382D and A459D). The crystal structures of the orthologous *E. coli* and *B. stearothermophilus* MutY proteins indicate that the mutated tyrosine at human residue 165 forms part of the pseudo-HhH domain and intercalates the DNA 5′ of the oxidised guanine: a critical step in 8-oxoG strand recognition [59, 64]. The equivalent mutant protein in *E. coli*, Y82C-MutY, displays minimal adenine glycosylase activity and Y165C-MUTYH has a reduced ability to complement *E. coli mutY*$^{-}$, as compared to the wild type enzyme [68]. G382D is also predicted to affect 8-oxoG recognition by disrupting the structure of a C-terminal turn that hydrogen bonds with the damaged strand [64]. In vitro studies of the corresponding *E. coli* mutant protein, G253D-MutY, revealed an 85% reduction in glycosylase activity with an 8-oxoG:A mispair and expression of the mouse mutant (G365D-Mutyh) in *Mutyh* null ES cells failed to complement the mutator phenotype or produce detectable glycosylase activity [69]. R227W, R231L and V232F lie near to or within the putative MSH6 binding domain and although none of these variants affect the physical interaction with MSH6, R227W- and R231L-MUTYH have severe defects in 8-oxoG:A binding and glycosylase activities, while V232F-MUTYH has reduced 8-oxoG:A binding and glycosylase activities [48, 70]. A459D-MUTYH has also been shown to have reduced repair activity [39].

7.5 Genetic Testing and Clinical Management of MAP

Genetic testing of *MUTYH* in patients with phenotypic features suggestive of MAP is essential in planning for the surveillance needs in the extended family. MAP must be distinguished from FAP and AFAP as it is the siblings, rather than offspring of MAP cases who are most likely to require further investigation. Genetic testing can be used to identify those siblings of MAP cases who are at risk and also to clarify the genetic status of spouses of those with biallelic mutations so that their offspring can be counselled accurately. Since polyp number may be very low (or even zero) in cases with CRC and biallelic *MUTYH* mutations, some have suggested wider testing for *MUTYH* among incident CRC cases [30].

We recommend annual or biennial colonoscopic surveillance for individuals with biallelic *MUTYH* mutations, commencing by 20 years of age. Although duodenal adenomas have been reported in some MAP patients, the case for upper gastrointestinal tract surveillance is unclear and there is currently no evidence for screening of other organs. Surgical options for colorectal disease need to be tailored to the individual patient, since tumour burden can apparently vary from a count of one to many hundreds; however, many patients will require surgery to remove the large bowel.

The health consequences of heterozygosity for inherited *MUTYH* mutations are unclear. Somatic inactivation of the wild type *MUTYH* allele in a colonic crypt stem cell in a heterozygote might lead to a mutator phenotype and predispose to CRC. Consistent with this possibility, some studies have identified more frequent chromosome 1p loss of heterozygosity (corresponding to the chromosomal location of *MUTYH*) in CRCs from carriers of germline *MUTYH* variants than in CRCs from non-carriers [25, 71]. A number of case-control association studies have been undertaken to investigate CRC risk in heterozygotes and although these have drawn different conclusions, meta-analysis suggests at most only a minor increase in CRC risk [72, 73].

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Chapter 8
Polymorphic Variation and Risk of Colorectal Cancer

Richard A. Hubner and Richard S. Houlston

Abstract Colorectal cancer (CRC) is the third commonest cancer worldwide after lung and breast cancer, and two-thirds of CRCs occur in developed countries [1, 2]. Despite recent advances in treatment the prognosis for CRC patients with advanced stage disease remains poor, and there is an urgent need for strategies to identify individuals with an increased CRC risk so that colonoscopic screening and chemoprevention can be directed to those who will obtain most benefit [1].

First-degree relatives of CRC patients have an approximately twofold increased risk of developing the disease themselves, and the risk increases with increasing number of affected family members and if CRC is diagnosed at a young age [3]. This familial aggregation may be due to shared environment, inherited factors, or a combination of both, but twin studies have provided convincing evidence that approximately a third of CRC can be ascribed to inherited factors [4]. Highly penetrant mutations have been characterised in the known CRC susceptibility genes *APC*, mismatch repair (MMR) genes, *STK11/LKB1*, *SMAD4*, and *MUTYH*, which respectively result in the syndromes of familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome, Peutz–Jeghers syndrome, juvenile polyposis syndrome, and *MUTYH*-associated polyposis (MAP). However, these syndromes together account for only about 5% of CRCs [5]. The nature of the remaining familial risk is unknown, but it is likely that a substantial proportion is conferred by a number of low-penetrance genetic variants with relatively high population frequency. Individually, these polymorphisms will be associated with only modest increases in risk, but when considered collectively they may confer substantial susceptibility [6]. Polymorphisms may influence CRC risk directly, may interact with each other or with relevant environmental exposures, and may influence the effectiveness of chemopreventive and chemotherapeutic agents. Identification of such low-penetrance colorectal susceptibility polymorphisms will not only permit more accurate determination of an individual's CRC risk and thus allow more effective

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application of screening and preventive strategies, but may also provide further insights into the molecular pathways involved in colorectal carcinogenesis, and aid the discovery of novel drugs for CRC prevention and treatment.

Keywords Polymorphic • Variation • Risk • Colorectal cancer

8.1 Distribution of Polymorphisms and Linkage Disequilibrium in the Human Genome

A polymorphism is a DNA sequence variation in which the less common (minor) allele has a population frequency of at least 1%. The frequency of the same polymorphism, however, may vary widely between different geographical populations and ethnic groups [7]. Deletions, insertions and tandem repeat sequences account for a small proportion of polymorphic variation, but single nucleotide polymorphisms (SNPs) are by far the most common form, with an estimated ten million SNPs occurring in the human genome and collectively accounting for over 90% of sequence variation [8]. They are distributed throughout the genome, occurring approximately every 100–300 base pairs but with marked regional differences, the vast majority lying in untranslated regions outside known genes [9].

Adjacent polymorphisms in the same chromosomal region are not inherited randomly but as a combination of alleles which form haplotype blocks [10]. This phenomenon is termed linkage disequilibrium (LD), and the strength of LD between two adjacent polymorphisms is dependent on the physical distance between them along the chromosome. Variants that are very close to each other are tightly linked due to the low probability of a meiotic recombination between them. In contrast, widely spaced polymorphisms will exhibit low LD since recombination during meiosis is more likely. The degree of LD between two adjacent polymorphisms cannot, however, be simply predicted by the chromosomal distance between them, since the pattern of LD within the human genome is not uniform [11, 12]. Distinct blocks of high LD occur, and are interspersed with regions in which LD breaks down rapidly [13].

LD underlies the principle of gene mapping by association analysis. LD between a marker allele and a disease susceptibility allele will result in both alleles being inherited together over many generations; thus the same marker allele will be detected in affected individuals from apparently unrelated families. Recombination between the marker and disease susceptibility allele will eventually dissipate the association (as can further mutational events), with the rate of decay being primarily dependent on the distance between the two alleles and the number of generations that has passed [14, 15]. The slowness of this decay, however, makes allelic association a useful tool. Additionally, the complexity of analysing a number of different SNPs within a particular gene or locus, can be significantly reduced if there is strong LD between them, since the genotype of all the SNPs within the haplotype block can be inferred from the genotyping of only one or a few marker

SNPs or 'tagging SNPs' [16]. Linkage disequilibrium can thus be further exploited in association studies by using tagging SNPs to reduce the number of SNPs that require genotyping, significantly lowering laboratory costs.

8.2 The Association Study Design for Identifying Low Penetrance Cancer Susceptibility Alleles

8.2.1 Linkage Versus Association

Highly penetrant cancer susceptibility alleles result in extensive pedigrees and are most readily localised through linkage studies in which genetic markers co-segregate with disease. Low penetrance alleles conferring more modest risks, typically twofold or less, will rarely cause multiple-case families and will not be identified efficiently through linkage strategies [17]. The search for low penetrance CRC susceptibility alleles has therefore focused on the association study design where the frequencies of candidate alleles are compared in CRC cases and cancer-free controls. A marker allele is said to be associated with a disease if the allele is found more frequently among cases than in the general population, or in a group of unaffected individuals. Association between a marker allele and disease can be a consequence of either a direct biological action of the marker allele, or linkage disequilibrium between the marker allele and a disease-causing allele [18]. The association study design is advantageous since large numbers of case and control samples may be readily obtained, providing adequate power to detect relatively small effects. To detect an allele with a population frequency of 10% conferring a twofold increased risk, linkage analysis would require about 10,000 affected sibling pairs whereas an association study would only require 500 unselected cases and 500 controls [19].

8.2.2 Enriching for Genetic Susceptibility

The population frequency of a putative susceptibility allele is an important consideration when designing an association study, since allele frequency markedly influences power to detect an association, and hence the required number of cases and controls (Fig. 8.1). Assuming two controls per case, 800 unselected cases would be required to achieve 90% power at a significance level of 0.01 to detect a dominant allele with a population frequency of 5% that confers a twofold increased risk [19]. In contrast, if the population frequency were only 1%, then 3,700 cases would be required to achieve the same level of statistical power. Thus the use of unselected cases in association studies is satisfactory for the evaluation of common alleles, but has limited power if the population frequency is less than 5%. Power can be increased in association studies, however, by selecting cases that are enriched

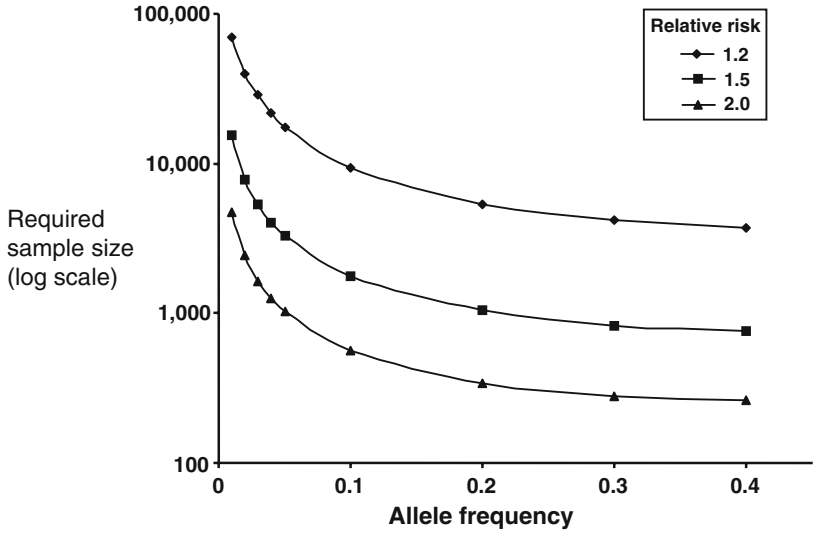

Fig. 8.1 Effect of allele frequency and relative risk on the required sample size to generate 90% power to show significant associations ($P=0.01$) for co-dominant susceptibility alleles, assuming one control per case

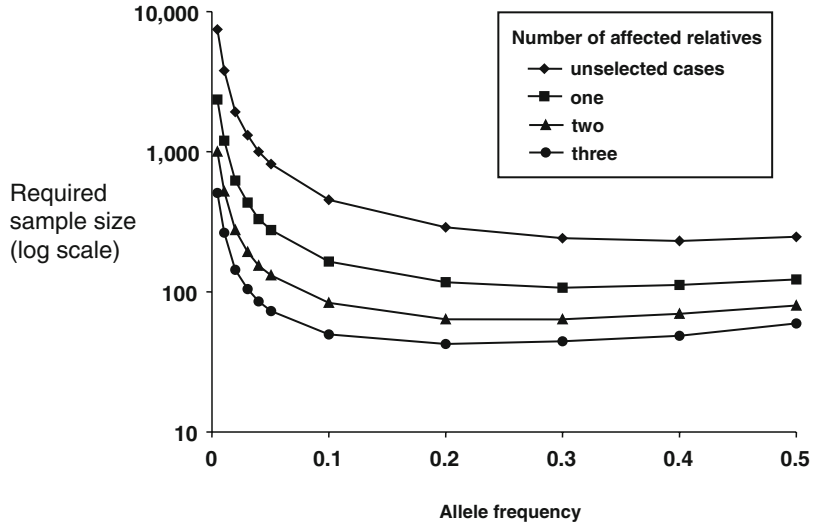

Fig. 8.2 Effect of affected family members on sample sizes required to generate 90% power to show significant associations ($P=0.01$) for co-dominant susceptibility alleles conferring a relative risk of two, assuming one control per case

for genetic susceptibility by virtue of a family history of cancer (Fig. 8.2). The sample size required is typically reduced by more than twofold if cases with an affected first-degree relative are selected, and by more than fourfold if cases with

two affected first-degree relatives are used [20, 21]. In the example above, the number of cases required would be reduced from 3,700 to 700 if cases with two affected first-degree relatives were selected.

8.2.3 Failure to Replicate Positive Associations

The relative ease of collecting DNA samples from unselected cases and controls and the extensive range of genetic variants that could plausibly be associated with cancer susceptibility has made association studies very popular. Few reported associations have been established beyond reasonable doubt, however, and in most instances, initially significant associations cannot be replicated in subsequent sample sets [22–24]. This is most likely due to type I errors, the spurious association of genetic variants with disease, compounded by publication bias [6]. Levels of statistical significance that are appropriate in other contexts ($P = 0.05$ or $P = 0.01$) may not be suitable for association studies, since the number of possible genetic polymorphisms that could be tested is very large and the prior probability that any particular polymorphism will be associated with disease is low, thus most variants achieving a modest level of statistical significance will be false positives [6]. The false-positive rate can be reduced by setting more stringent levels of statistical significance or, at least in principle, by improving the selection of candidate polymorphisms to increase the prior probability of association.

A second possible explanation for the failure to replicate initially positive associations is inadequate statistical power in the replication study, leading to type II errors or false negatives [19]. For example, fewer than 40% of the colorectal association studies reviewed by Houlston and Tomlinson [24] had 80% or more power to detect a twofold difference in risk at the 0.05 significance level. Very large sample sizes, in the order of thousands or tens of thousands, are required to identify and confirm, or conclusively refute, genetic variants conferring modest CRC susceptibility.

8.2.4 Population Stratification

A further common explanation for spurious association is population stratification, the existence of multiple population subgroups in what was assumed to be a homogeneous population in which allele frequencies vary between the different subgroups [25]. If cases and controls are selected differentially from these subgroups – for example, if a disease is more common in one ethic group – then allelic association will occur in the absence of a true biological association. One way to circumvent this problem is to use family-based approaches, such as the transmission disequilibrium test (TDT), which assess the evidence for preferential transmission of one allele over another in heterozygous parents. Outside the context of childhood cancers,

this approach is not suitable, however, since it involves genotyping the affected case and both parents; the latter are often not available, and the use of other family members severely reduces power [26]. In reality, there are few actual examples to support the suggestion that population stratification is a frequent cause of non-replicable associations, indicating that this problem has probably been overemphasised and that other factors such as type I errors and publication bias are more important [7]. Alternatively, failure to replicate associations may occur if there is genuine heterogeneity in risk in different populations. This could occur if there were population differences in LD patterns, allele frequencies of interacting genes, or interacting environmental exposures [6].

8.3 Direct Association Studies for CRC Susceptibility Polymorphism Discovery

Most of the known disease alleles in Mendelian cancer syndromes are variants within coding regions that result in protein truncation and hence total or very severe loss of function [8]. 95% of germline mutations in the *APC* gene giving rise to the colorectal cancer susceptibility syndrome familial adenomatous polyposis (FAP), for example, are protein truncating [27]. It has been argued that low penetrance cancer susceptibility alleles are similarly most likely to be coding variants, and the majority of analyses performed to date have been direct association studies which focus on polymorphisms that are thought to alter protein function or gene expression [17]. The analysis of functional variants has the potential to be a powerful method of cancer gene discovery since the number of common coding polymorphisms is only a fraction of all polymorphisms. It is estimated that there are 50,000–250,000 polymorphisms which confer a biological effect, most of which are distributed in and around the 30,000 genes [7]. Most direct CRC association studies have focused on putatively functional polymorphisms in genes that encode proteins thought to be relevant to colorectal carcinogenesis. Examples of such 'candidate genes' include carcinogen metabolism genes, genes involved in folate metabolism, colonic microenvironment modifier genes, tumour suppressor genes and oncogenes, genes involved in inflammation, and genes known to harbour high penetrance CRC-causing mutations.

8.3.1 Carcinogen Metabolism Genes

The colonic epithelium is exposed to dietary carcinogens such as heterocyclic amines (HAAs) in cooked meat [28]. The metabolic activation of these chemical carcinogens, which allows them to bind DNA, is mediated by the phase-I and phase-II enzymes cytochrome P450A1 (*CYP1A1*) and *N*-acetyl transferases (NAT) 1 and 2 [29]. The *CYP1A1* enzyme also activates polycyclic aromatic hydrocarbons

(PAHs) found in tobacco smoke [30]. Conversely, HAAs and PAHs are detoxified by glutathione-S transferase (GST) enzymes [31].

Polymorphisms in the genes coding for these enzymes have been demonstrated to influence enzyme activity, which may result in altered carcinogen exposure and hence differential CRC risk [32]. For example, individuals with a fast acetylator phenotype on the basis of their *NAT2*4* allele carrier status have been reported to have an increased CRC risk, as have individuals homozygous for null (non-functional) *GSTM1* and *GSTT1* alleles (Table 8.1) [24, 33]. The evidence for an influence of other carcinogen metabolism polymorphisms on CRC risk, however, is unclear [33].

8.3.2 Genes Involved in Folate Metabolism

Folate metabolism impacts on both DNA methylation and DNA synthesis and repair, and aberrations of both these processes are known to be important in colorectal carcinogenesis [34]. Epidemiological studies lend further support for a role of folate metabolism in CRC development with high folate intake individuals generally showing a reduced CRC incidence [35]. The enzymes involved in folate metabolism control the flow of one-carbon moieties towards methylation or DNA synthesis, making functional polymorphisms in the genes coding for these enzymes attractive CRC susceptibility candidates. The methylenetetrahydrofolate reductase (*MTHFR*) enzyme occupies a pivotal position within the folate metabolism pathway and is considered to be the rate-limiting enzyme (Fig. 8.3) [34]. The C677T polymorphism in the *MTHFR* gene is known to result in reduced enzyme function with homozygote carriers of the 677T allele having 35% of normal enzyme activity, and a second variant, A1298C, also confers reduced activity [36, 37]. The relationship between *MTHFR* C677T genotype and CRC susceptibility has been investigated in a number of studies, and a recent meta-analysis indicated a significantly reduced risk in homozygous carriers of the variant allele (see below) [38]. The A1298C variant has been investigated in fewer studies but also appears to be associated with reduced CRC risk [39]. A number of other variants in folate metabolism genes have also been investigated and may confer altered CRC risk [40–46].

8.3.3 Colonic Microenvironment Modifiers

The colonic microenvironment is modified by bile acid secretion [47], and the apolipoprotein E (*APOE*) enzyme is involved in the regulation of cholesterol and bile acid metabolism [48]. Three common alleles of the *APOE* gene, ε2, ε3, and ε4, influence serum total and very low density lipoprotein cholesterol clearance, and faecal bile acid output is lower in individuals with the ε4 allele [49]. Carriers of the ε4 allele have been reported to be at reduced CRC risk [33]. The *PLA2G2A* gene encodes secretory phospholipase A2 which is involved in the synthesis of prostaglandins. Polymorphisms

Table 8.1 Summary of pooled analyses of studies investigating associations between polymorphism genotypes and colorectal cancer risk

Polymorphism	Number of studies (total cases)	Ethnicity	Allele frequency	Risk group	Pooled OR (95% CI)	References
Carcinogen metabolism						
CYP1A1 MspI RFLP	3 (398)	Caucasian	0.11	Variant homozygotes	0.81 (0.23–2.82)	[33]
CYP1A1 A462G	3 (460)	Mixed	0.14	Variant homozygotes	0.96 (0.48–1.95)	[33]
CYP2D6 deletion	2 (309)	Caucasian	0.20	Deletion carriers	1.12 (0.90–1.40)	[33]
CYP2E1 G1259C	2 (312)	Caucasian	0.05	Variant carriers	1.53 (0.97–2.42)	[33]
GSTM1 deletion	10 (3,460)	Mixed	0.50	Deletion homozygotes	1.10 (0.99–1.21)	[24]
GSTP1 codon 105	5 (693)	Mixed	0.31	Variant homozygotes	0.91 (0.36–2.31)	[33]
GSTP1 codon 114	2 (327)	Mixed	0.08	Variant homozygotes	0.45 (0.09–2.36)	[33]
GSTT1 deletion	11 (1,490)	Mixed	0.21	Deletion homozygotes	1.37 (1.17–1.60)	[33]
NAT1 genotype	4 (587)	Mixed	0.45	Fast acetylators	1.09 (0.85–1.40)	[33]
NAT2 phenotype	4 (304)	Mixed	0.37	Fast acetylators	1.70 (1.23–2.37)	[24]
NAT2 genotype	11 (3,690)	Mixed	0.49	Fast acetylators	1.03 (0.93–1.14)	[24]
NQ01 C609T	5 (1,637)	Caucasian	0.35	Variant carriers	1.18 (1.02–1.35)	[102]
Methylation						
CBS 844ins68	3 (600)	Mixed	0.03	Variant carriers	0.69 (0.46–1.03)	UP[1]
MTHFR C677T	25 (12,243)	Mixed	0.35	Variant homozygotes	0.83 (0.75–0.93)	[38]
MTHFR A1298C	11 (4,764)	Mixed	0.28	Variant homozygotes	0.80 (0.69–0.94)	[39]
MTR A2756G	5 (3,281)	Mixed	0.18	Variant carriers	0.89 (0.81–0.99)	UP[2]
MTRR A66G	2 (679)	Mixed	0.31	Variant homozygotes	1.67 (1.17–2.41)	UP[3]

Microenvironment modifiers						
APOE	2 (289)	Mixed	0.17	E4 allele carriers	0.76 (0.56–1.03)	[33]
Oncogenes and tumour suppressor genes						
HRAS-1 VNTR	5 (394)	Caucasian	0.02	Rare allele carriers	2.50 (1.54–4.05)	[24]
L-*myc*	4 (324)	Mixed	0.51	Small allele homozygotes	1.09 (0.69–1.72)	[33]
Tp53 Exon 4 (codon 72)	2 (239)	Mixed	0.33	Variant homozygotes	1.34 (0.83–2.14)	[33]
APC I1307K	3 (670)	Ashkenazim	0.06	Variant carriers	1.58 (1.21–2.07)	[24]
Inflammatory response						
TNFα TNFa	2 (195)	Caucasian	0.22	a2 allele carriers	2.02 (1.51–2.71)	[33]
			0.07	a5 allele carriers	0.37 (0.18–0.72)	
			0.05	a13 allele carriers	0.36 (0.16–0.81)	
TNFα −308G>A	2 (167)	Asian	0.10	Heterozygotes	0.83 (0.51–1.34)	[33]
				Variant homozygotes	0.61 (0.07–5.47)	
Other genes						
HFE C282Y	3 (517)	Caucasian	0.06	Heterozygotes	0.85 (0.57–1.26)	[33]
				Variant homozygotes	0.60 (0.11–3.28)	
ALDH2 codon 487	2 (316)	Asian	0.07	Heterozygotes	3.00 (2.15–4.18)	[33]
				Variant homozygotes	12.8 (3.7–44.9)	
VDR *BsmI* RFLP	3 (766)	Mixed	0.41	Variant homozygotes	0.89 (0.66–1.19)	[33]

UP[1]: unpublished pooled analysis of data from three published studies [40–42]
UP[2]: unpublished pooled analysis of data from two published studies [41–45]
UP[3]: unpublished pooled analysis of data from two published studies [41, 46]

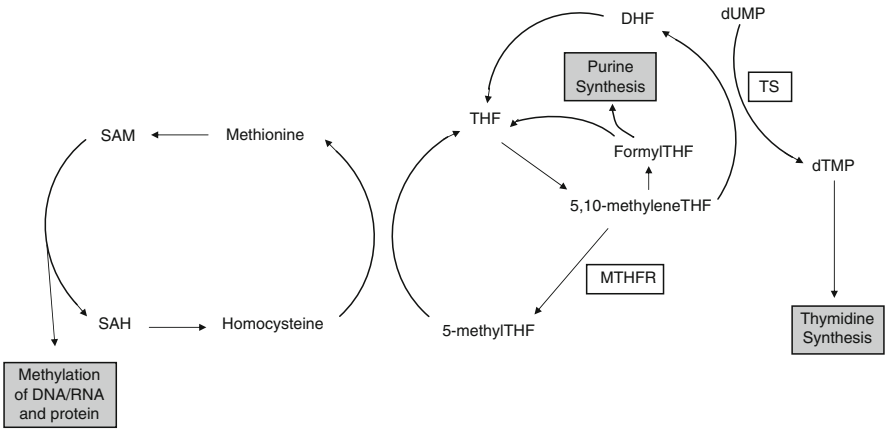

Fig. 8.3 Schematic representation of folate metabolism. *MTHFR* methylenetetrahydrofolate reductase, *TS* thymidylate synthase, *THF* tetrahydrofolate, *DHF* dihydrofolate, *dUMP* deoxyuridine monophosphate, *dTMP* deoxythymidine monophoshate, *SAM* S-adenosylmethionine, *SAH* S-adenosylhomocysteine

of the *PLA2G2A* gene dramatically increase the number of intestinal polyps in a mouse model of CRC making them attractive CRC susceptibility candidates [50]. A study in humans, however, failed to demonstrate an association with CRC risk [51].

8.3.4 Tumour Suppressor Genes and Oncogenes

The *Harvey ras-1* variable number tandem repeat polymorphism (*HRAS1*–VNTR) is a mini-satellite located 1 kilo base downstream of the *HRAS1* proto-oncogene. Over 30 alleles of the *HRAS1*–VNTR have been described with the four most common representing over 90%, and the remainder grouped as 'rare alleles' [52]. A meta-analysis of five studies investigating the relationship between *HRAS1*–VNTR genotype and CRC risk indicated that carriers of rare alleles have a 2.5-fold increased CRC risk [24]. The *HRAS1*–VNTR has been shown to modulate the expression of nearby genes interacting with transcriptional regulatory elements such as the rel/NF-κB family, and this may be the mechanism through which rare alleles predispose to CRC. Polymorphisms of the *TP53* tumour suppressor gene, which plays a role in protecting against replication of damaged DNA [53] and is somatically mutated in CRC [54], have also been studied in relation to CRC risk, but no robust associations identified [33].

8.3.5 Genes Involved in Inflammation

Epidemiological studies indicate that regular use of anti-inflammatory drugs such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) is associated with

reduced CRC risk, whilst ulcerative colitis, a chronic inflammatory disease affecting the large bowel mucosa, is associated with an ~10-fold elevated CRC risk [55]. Polymorphisms in the vicinity of the gene coding for the pro-inflammatory cytokine tumour necrosis factor-α (*TNF*-α) show associations with CRC risk [33]. The *TNFA* polymorphism, for example, has fourteen different alleles (a1–a14), which may result in altered *TNF*-α production, and an increased CRC risk has been reported in carriers of the a2 allele, whilst a5 and a11 allele carriers show a reduced risk [33].

Caspases are enzymes involved in regulating the balance between activation and apoptosis of anti-tumour T lymphocytes which have a pivotal role in immune surveillance of cancer cells [56]. Recently, a six-nucleotide deletion polymorphism in the promoter region of the gene coding for the caspase-8 enzyme, *CASP8* −652 6N del, was reported to result in lower T lymphocyte caspase-8 activity and activation-induced cell death upon stimulation with cancer cell antigens [57]. Homozygote carriers of the polymorphism were at 50% reduced risk of CRC, and also showed risk reductions in other tumour types including breast, lung, oesophageal, and gastric cancers [57].

8.3.6 Genes Harbouring High Penetrance CRC Susceptibility Mutations

It is entirely plausible that, in addition to highly penetrant truncating mutations, polymorphisms in genes such as *APC*, *MLH1*, *MSH2*, and *MUTYH*, may confer susceptibility to CRC. The *APC* I1307K variant, for example, is found in ~6% of the Ashkenazim population in which it confers an ~1.6-fold increased CRC risk [24]. The mechanism by which this sequence variant, which is rare in other ethnic groups, predisposes to CRC is thought to be the creation of a poly-(A_8) tract, instead of the normal A_3TA_4 sequence, which increases the rate of somatic *APC* mutations. The *MLH1* D132H polymorphism is found in 1.3% of the Israeli population and confers an ~5-fold increased CRC risk, and interestingly predisposes to predominantly microsatellite stable (MSS) CRC [58]. This variant maps to an evolutionarily conserved β-hairpin structure that is part of the ATP binding and hydrolysis domain of the *MLH1* protein, and causes instability between two other residues within the domain. The importance of the β-hairpin is demonstrated by the *MLH1* A128P mutation which disrupts the β-hairpin formation completely and is associated with HNPCC [58]. The D132H polymorphism results in attenuation rather than complete disruption of ATPase activity which may account for the differing tumour phenotype.

Recently, a promoter polymorphism, −93G>A, in the *MLH1* gene region was found to predispose to CRC exhibiting the microsatellite instability (MSI) phenotype [59]. Although the functional effects of this variant are unknown, it may result in altered *MLH1* expression which could plausibly lead to disruption of mismatch repair and hence increased MSI CRC risk. Demonstrating causality for such polymorphisms may be difficult, however, if they show LD with high-penetrance CRC causing mutations.

8.3.7 Other Genes

Polymorphisms in a large number of other candidate genes have been investigated for association with CRC risk. Those showing positive associations in single studies are listed in Table 8.2. Many have shown associations only in small sample sets, however, and require confirmation in multiple, large, independent studies before they can be unambiguously asserted as low-penetrance susceptibility alleles.

8.4 Meta-Analyses of Direct Association Studies

The assembly of adequately sized sample sets has been a frequent obstacle to identifying low penetrance CRC susceptibility alleles. Pooling of data from a number of different studies that have analysed the same polymorphism in independent sample sets has therefore been a popular method of generating adequate statistical power [22, 24, 33]. For example, a recent updated meta-analysis of studies investigating the relationship between *MTHFR* C677T genotype and CRC risk indicated that, compared to the homozygous wild-type genotype, the *MTHFR* 677TT genotype was associated with a 17% reduction in CRC risk (OR 0.83; 95% confidence interval (CI): 0.75–0.93) [38]. Although an OR of 0.83 is modest, because of the high frequency of the wild-type allele conferring an increased risk (0.68 in Caucasian populations), this translates into a relatively high population attributable risk, and it was estimated that *MTHFR* C677T genotype contributed to approximately 15% (95% CI: 9–22%) of the total incidence of CRC. It is noteworthy that only a few of the studies included in this meta-analysis individually showed a significant association with CRC risk, and pooling of data from over 12,000 individuals with CRC was required to demonstrate a significant association. This illustrates the order of magnitude of the sample sizes required to generate adequate statistical power to reliably identify low penetrance susceptibility alleles. Other examples of associations between polymorphism genotype and CRC susceptibility with evidence from pooled analyses are listed in Table 8.1.

When considering such pooled analyses, it is important to identify methodological issues that may affect their reliability. First, an extensive search of all studies potentially suitable for inclusion in the pooled analysis should be performed, and few, if any, exclusion criteria should be applied to avoid ascertainment bias. Where possible, authors should be contacted directly if the relevant data has not been presented in publications. Second, evidence of significant heterogeneity between the individual studies included in the analysis makes interpretation of the pooled estimate problematic [60]. Where there is evidence of such between-study heterogeneity, attempts should be made to identify potential sources, such as differences in study design, in particular the use of hospital-based rather than population-based control subjects, differences in ethnicity or geographic location of study subjects,

Table 8.2 Summary of polymorphisms showing associations with colorectal cancer risk in single studies

Polymorphism	Number of cases/ controls	Ethnicity	Allele Frequency	Risk group	OR (95%CI)	References
ARLTS1 C148A	328/515	Caucasian	0.48	Variant allele carriers	1.59 (1.13–2.23)	[103]
CASP8 –652del6	930/4,972	Asian	0.20	Heterozygotes	0.80 (0.65–0.99)	[57]
				Variant homozygotes	0.50 (0.31–0.79)	
CDH1 –347insA	260/147	Asian	0.14	Variant allele carriers	1.97 (1.07–3.61)	[104]
CHEK2 I157T	837/1,885	Caucasian	0.05	Variant allele carriers	1.4 (1.0–2.0)	[105]
DRD2 –141delC	370/327	Caucasian	0.04	Variant allele carriers	2.28 (1.38–3.76)	[106]
ERbeta CA repeat	1,580/1,968	Mixed	0.20	Female ≥ 25 repeat homozygotes	2.13 (1.24–3.64)	[107]
EXO1 T439M	102/110	Asian	0.21	Variant allele carriers	2.37 (1.23–4.56)	[108]
GSTT2 –537G>A	436/568	Asian	0.42	Variant allele carriers	1.37 (1.04–1.81)	[109]
MLH1 D132H	1,299/1,395	Mixed	0.01	Variant allele carriers	4.60 (1.7–12.30)	[58]
MSH3 T1036A	237/2,189	Caucasian	0.29	Variant homozygotes	1.65 (1.01–2.70)	[110]
MMP-1 –1607 1G/2G	101/127	Asian	0.75	2G homozygotes	2.01 (1.22–3.53)	[111]
MMP-1 –1607 1G/2G	127/208	Caucasian	0.49	2G carriers	1.41 (1.02–1.96)	[112]
MMP-3 6A	101/127	Asian	0.88	6A homozygotes	2.11 (1.17–3.82)	[111]
IGFBP-3 G2133C	817/1,995	Mixed	0.53	Variant allele carriers	1.32 (1.07–1.62)	[113]
IGF1 CA repeat	782/503	Mixed	0.41	non-19 repeat carriers	1.3 (1.0–1.6)	[114]
IL6 –174G>C	377/326	Caucasian	0.31	Variant allele carriers	1.53 (1.12–2.09)	[115]
IL8 –251T>A	377/326	Caucasian	0.43	Variant allele carriers	0.70 (0.50–0.99)	[115]
IRS1 G972R	1,346/1,544	Mixed	0.06	Variant allele carriers	1.40 (1.10–1.90)	[116]
MGMT I143V	197/2,500	Mixed	0.01	Variant allele carriers	0.52 (0.33–0.80)	[117]
OGG1 S326C	377/329	Caucasian	0.19	Variant homozygotes	2.30 (1.05–5.09)	[118]
PPARG P12A	377/326	Caucasian	0.11	Variant allele carriers	0.56 (0.37–0.85)	[115]
PPARG P12A	362/1,164	Asian	0.04	Variant allele carriers	0.53 (0.30–0.92)	[119]

(continued)

Table 8.2 (continued)

Polymorphism	Number of cases/ controls	Ethnicity	Allele Frequency	Risk group	OR (95%CI)	References
SMYD3 E2F-1 repeat	175/365	Asian	0.21	Variant allele carriers	2.58 (1.68–3.94)	[120]
TGFβ1 −509C>T	152/250	Asian	0.35	Variant allele carriers	0.59 (0.28–0.92)	[121]
TS 5′ UTR 28bp repeat	270/454	Caucasian	0.47	2R homozygotes	0.59 (0.36–0.98)	[122]
TS 1494del6	208/675	Caucasian	0.33	del6 homozygotes	1.4 (1.0–2.0)	[123]
VDR FokI RFLP	1,811/1,451	Mixed	0.35	f allele carriers	1.15 (1.03–1.28)	[124]
VDR FokI RFLP	217/890	Asian	0.47	ff homozygotes	1.84 (1.15–2.94)	[125]
XRCC3 T241M	140/280	Asian	0.02	Variant allele carriers	3.13 (1.41–6.95)	[126]

and the methods of genotyping employed [61]. Third, an assessment of publication bias should be made, as significant publication bias means that the pooled estimate is unlikely to reflect the true influence of the genetic variant under study [62]. Despite these potential methodological problems, carefully designed pooled analyses remain a useful tool for confirming or refuting potential CRC susceptibility polymorphisms.

8.5 Indirect Association Studies

A major disadvantage of the direct association approach is that it relies on existing knowledge to select candidate genes, identify potentially functional polymorphisms within these genes through database searching, and accurately predict their functional effects. The identification of large numbers of SNPs across the human genome has allowed association studies to progress from the analysis of a small number of specific candidate polymorphisms, to assessing a much greater proportion of the genetic variation within a particular gene or gene region to detect any allelic association. Such indirect association studies rely on LD between multiple SNPs across a small region, allowing analysis of all SNPs within the LD block through the genotyping of one or a few tagging SNPs. With indirect association studies it is assumed that any cancer causing SNP within the region is unlikely to be analysed directly, rather SNPs in the same LD block will be genotyped, and hence show association with disease. The recent rapid advances in our knowledge of polymorphic variation, and the availability of this information in public databases, has allowed the development of methods and software to select SNPs spanning gene regions such that at least one SNP per LD block is chosen for analysis [63, 64]. This set of tagging SNPs can then be genotyped in a series of cases and controls to test for association. If an association is found, the component SNPs within the LD block should be examined to determine the causal variant, a process that may involve genotyping of additional SNPs to better define the haplotype structure.

8.6 Genome-Wide Association Studies

Until very recently, extension of the indirect association approach to evaluation of the entire human genome was prohibitively expensive, due to the need to analyse several hundred thousand SNPs to achieve adequate coverage. The development of analytical platforms capable of parallel processing, however, now allows simultaneous genotyping of 500,000 SNPs on a single array at a cost of only a few hundred dollars per sample, making genome-wide association (GWA) studies economically feasible. Evaluation of such large numbers of SNPs presents new problems in terms of interpretation, in particular in relation to thresholds for statistical significance. If 500,000 SNPs are genotyped, a P value of 0.01 for statistical significance will

result in 5,000 SNPs showing an association by chance alone. Although setting lower thresholds for significance or compensating for multiple tests can help to address this issue, the employment of a multi-stage study design may be a more efficient solution to this problem [6]. In the first stage of such studies, a set of cases and controls are genotyped for the entire set of SNPs. Polymorphisms that are below a nominal level of significance are then selected for genotyping in a second, larger series of cases and controls to identify the true-positives and false-positives from the first stage. The SNPs that still remain significantly associated may then be tested in additional sample sets where available.

Genome-wide association studies have unequivocally identified ten CRC susceptibility loci; six by individual GWA studies (8q24 [65, 66], 8q23.3 [67], 10p14 [67], 11q23 [68], 15q13 [69], and 18q21 [68, 70]), and a further four through a meta-analysis of data from two independent GWA studies (14q22, 16q22.1, 19q13.1, and 20p12.3) [71]. The relative risks (RRs) conferred by these loci are low, varying from ~1.3 to 1.1, and in most cases the minor allele is associated with an increased risk of CRC in a dose-dependent manner with a higher risk in homozygous than heterozygous carriers. Although the contribution of each of the ten loci to the familial risk of CRC is less than 1%, conservative estimates indicate that collectively they may account for ~6% [71]. Intriguingly, most of the loci map to regions of the genome that do not contain known genes and thus cannot be accounted for by linkage with coding variants, but may instead confer susceptibility through as yet unknown mechanisms resulting in altered gene-expression. Furthermore, the variant at 8q24 also confers susceptibility to prostate and ovarian cancers indicating pleiotropic effects [72, 73].

The GWA studies conducted to date have had high power to detect variants with minor allele frequency > 0.2 and which confer RRs of 1.2 or greater, so it is unlikely that further CRC susceptibility variants with similar effects remain undiscovered. In contrast, there has been low power to detect variants with smaller effects or those with lower minor allele frequencies, and thus it is likely that many more such susceptibility loci exist. Identification of these variants will be dependent upon further GWAs with even greater sample sizes, and which genotype larger numbers of SNPs.

8.7 Gene–Environment and Gene–Gene Interactions

Functional polymorphisms in genes coding for enzymes involved in drug or carcinogen metabolism may not directly influence cancer risk, but may modify the effect of environmental factors and in this manner alter cancer susceptibility in exposed individuals. Such effects have been termed gene–environment interactions, and they may not only confer cancer susceptibility but may also modify an individual's response to anticancer therapies [74]. Investigation of gene–environment interactions can be included in association studies by appropriate measurement of the environmental factor of interest, and analysis of the effect of genotype on disease risk following stratification by exposure.

Examples of gene–environment interactions include the *MTHFR* C677T polymorphism and folate status, and variant uridine diphosphate glucuronosyltransferase 1A6 (*UGT1A6*), prostaglandin H synthase 1 (*PTGS1*, or cyclooxygenase1 (*COX1*)) and *PTGS2* genotypes and aspirin use [75, 76]. In these examples, the genetic variants have been demonstrated to interact with the environmental exposure to determine colorectal neoplasia risk. Individuals with the less common *MTHFR* 677TT genotype are at reduced risk of CRC if they have adequate folate status, but at paradoxically increased CRC risk if they have inadequate folate intake [75, 77]. The *UGT1A6* enzyme participates in the metabolism of aspirin and other non-steroidal anti-inflammatory drugs, and functional polymorphisms of *UGT1A6* which result in reduced enzyme function, have been reported to modify the protective effect of aspirin on colorectal adenoma (CRA) risk [76]. Individuals with wild-type *UGT1A6* genotypes did not gain benefit from taking aspirin, whilst those with the reduced function genotypes had a lower risk of developing CRA but only if they were exposed to long-term aspirin. The *PTGS1* and *PTGS2* enzymes catalyse prostaglandin synthesis, and play important roles in regulating both constitutive and reactive inflammation [78]. Both enzymes are inhibited by aspirin and other NSAIDs, and this inhibition is thought to mediate at least part of the protective effect of these drugs in colorectal carcinogenesis [79]. In a case-control study of colorectal adenoma (CRA) patients, the genotype for the *PTGS1* 50C>T polymorphism had no main effect on risk, but homozygote carriers of the *PTGS1* 50C allele showed a reduced CRA risk if they reported regular aspirin or NSAID use, whilst individuals with one or two *PTGS1* 50T alleles showed no benefit from NSAID use [80]. Similarly, in the same group of CRA cases and control subjects, no direct influence of *PTGS2* −765G>C genotype on risk of developing CRA was reported, but a significant interaction between genotype and aspirin or NSAID use was noted, with the benefits of NSAIDs being confined to carriers of *PTGS2* −765G alleles [81].

Gene–gene interactions occur when the effect of one genetic variant is modified by the presence or absence of a further variant or variants, either in the same gene or in genes coding for other enzymes within the same metabolic pathway. As with gene–environment interactions, gene–gene interactions imply that susceptibility to CRC will be conferred by such polymorphisms only in subgroups of the population defined by genotype of the interacting variant. Alternatively, a polymorphism may confer low-level susceptibility when the population is considered as a whole, but a higher level of susceptibility in the relevant subgroup. For example, a case-control study of CRA risk has reported a gene–gene interaction between the thymidylate synthase (*TS*) 28 base-pair enhancer repeat (*TSER*) polymorphism and the *MTHFR* C677T polymorphism [82]. The increased risk associated with the *MTHFR* 677TT genotype in low folate intake individuals was confined to those with *TSER* 3R/3R genotype. The *TS* and *MTHFR* enzymes share the same substrate, 5,10-methylenetetrahydrofolate, thus an interaction between polymorphisms in these two genes has biological plausibility (Fig. 8.3).

Attempts to identify further gene–environment and gene–gene interactions have often adopted the ‘candidate-pathway’ approach to CRC association studies, where multiple polymorphisms in a number of genes involved in a particular metabolic

pathway thought to be involved in colorectal carcinogenesis are genotyped, with accurate measurement of appropriate environmental exposures [83, 84]. The only caveat is that adequate assessment of interactions requires sample sizes a further order of magnitude greater than those required for investigation of the main effects of either genotype of environmental exposure separately [74].

8.8 Polymorphisms and Efficacy of Chemopreventive and Chemotherapeutic Drugs

Germline polymorphisms may not only confer altered cancer susceptibility, but may also account, at least in part, for the wide inter-patient variation in clinical response and toxicity to both conventional and novel cancer preventive and therapeutic agents. This is particularly likely with polymorphisms influencing metabolic pathways responsible for drug activation and inactivation. For example, aspirin is known to have chemopreventive activity in colorectal neoplasia as evidenced by reduced colorectal adenoma recurrence in regular aspirin users [85, 86], and a polymorphism in the ornithine decarboxylase (*ODC*) gene, G316A, has been shown to influence the effectiveness of aspirin in this setting [87, 88]. Both aspirin and *ODC* G316A genotype act independently to reduce tissue levels of polyamines, which are themselves associated with carcinogenesis, and in two CRA recurrence studies aspirin use was only effective in reducing recurrence in carriers of variant *ODC* G316A alleles [87, 88].

Irinotecan is a topoisomerase I inhibitor used in the treatment of metastatic CRC. SN38 is the active metabolite of irinotecan and is inactivated by glucuronidation by the uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) enzyme, which is the same enzyme that conjugates bilirubin [89]. Polymorphisms of the *UGT1A1* gene, in particular the *UGT1A1*28* variant characterised by an extra TA repeat in the promoter region of the gene, have been associated with reduced SN38 glucuronidation and hence investigated for a role in irinotecan toxicity [90]. Homozygosity for the *UGT1A1*28* allele occurs in ~10% of Caucasians and is also the commonest cause of Gilbert's syndrome [91]. The most common dose-limiting toxicities associated with irinotecan therapy are diarrhoea and neutropenia, which are most commonly observed with a weekly and 3-weekly schedule, respectively [92]. Initial small studies indicated that the toxicity of irinotecan, when used either as a single-agent or in combination with other agents, was related to *UGT1A1*28* genotype, with heterozygote and homozygote carriers experiencing increased toxicity, in particular neutropenia [93–95]. A recent large prospective study of irinotecan used in combination with 5-fluorouracil (5-FU) and leucovorin as first-line treatment for metastatic CRC, however, showed a less convincing relationship between toxicity and *UGT1A1*28* genotype, with only neutropenia following the first cycle of administration showing a significant association [96]. This illustrates the need for adequately sized prospective studies to fully investigate the potential roles of polymorphisms in determining response and toxicity to chemotherapy.

Other examples of polymorphisms with evidence for a potential role in determining the efficacy of CRC therapies are the thymidylate synthase *TSER* and 1494del6 variants and 5-FU [97, 98], the *MTHFR* C677T and A1298C polymorphisms and 5-FU [99], the *ERCC1* C118T polymorphism and combined oxaliplatin and 5-FU [100], and the *CCND1* A870G polymorphism and cetuximab [101]. To date, however, there is insufficient data to allow tailored treatments or dose adjustments in clinical practice based on these polymorphism genotypes.

8.9 Conclusions

A significant proportion of inherited susceptibility to CRC remains unaccounted for, and it is likely that polymorphisms will be responsible for an as yet unknown fraction of this risk. Alternatively, multiple rare variants could account for the remaining susceptibility, identification of which will require large-scale, high-throughput resequencing. The lessons learned from early association studies will aid the design of future studies, and, in particular, the assembly of multiple, large sample sets will generate the required statistical power to reliably identify low penetrance susceptibility polymorphisms. Accurate assessment of relevant dietary and other environmental factors in the same study subjects will also aid investigation of gene–environment interactions. The advances in SNP genotyping technology have made genome-wide association studies a reality, and have resulted in the identification of a number of polymorphic variants conferring unequivocal CRC susceptibility, in addition to providing insights into the nature of the remaining familial excess risk.

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Part III
Pathology

Chapter 9
The Pathologist and the Phenotype of Hereditary Colorectal Cancer

Jeremy R. Jass

Abstract The aim of this review is not to provide a morphological description of each form of colorectal cancer family syndrome. The aim is to explain to a readership that is mainly from a non-pathology background why, when faced with a tissue sample or surgical specimen, it is extremely difficult to extract information that is comprehensive and maximizes the potential for informing the clinical and basic science researcher as well as guiding clinical management. The usual description of a colorectal tumor focuses on its histogenetic type. However, the microscopic appearances of polyps and cancers in cancer family syndromes frequently differ from the appearances of their sporadic counterparts. Even before considering these differences, it is necessary to deconstruct the formal description of biopsied or resected surgical specimens into its basic elements. With respect to polyps, the parameters in question include size, macroscopic appearances, number, anatomic location, and even the synthesis of findings accumulating over time. In critically analyzing these parameters, including the mechanisms underlying their marked phenotypic variation, the full scale of task faced by the pathologist is brought into focus.

Keywords Colon • Cancer • Pathology • Phenotype • Diagnosis

9.1 Linking Morphology, Behavior, and Genotype: The Central Challenge

Hereditary colorectal cancer can be broadly subdivided into two subtypes: those associated with numerous colorectal polyps and those in which polyps are present but in small numbers only. The most well-studied example of the former is familial

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adenomatous polyposis (FAP) [1]. In FAP, each adenoma has a limited propensity for malignant transformation. However, because affected subjects have many hundreds, if not thousands, of colorectal adenomas, it is inevitable that one or more adenoma will have become malignant by the time the subject reaches middle age. By contrast, Lynch syndrome or hereditary non-polyposis colorectal cancer is associated with very small numbers of pre-cancerous adenomas [2]. In a series of 22 adenoma-positive patients with Lynch syndrome, most subjects had only one adenoma and only three patients had the maximum of three adenomas [3]. Morphologically, Lynch syndrome adenomas do not differ greatly from the adenomas in FAP. Yet there is a strong likelihood that each Lynch syndrome adenoma will not only progress to colorectal cancer (CRC) but will do so within a short timeframe [4]. This rapid evolution may be appreciated when patients with a negative screening colonoscopy develop a CRC before the next screening examination (an interval cancer) [5]. It has been noted that colorectal adenomas in Lynch syndrome are more likely to show features linked to neoplastic progression. These features include large size, villous architecture, and high-grade dysplasia [6]. However, these features are not uncommon in sporadic adenomas and cannot be used by the anatomic pathologist diagnose Lynch syndrome.

The preceding brief outline of the two most well-studied forms of hereditary CRC exposes the diagnostic limitations of morphologic assessment in isolation. Anatomic pathology is a highly visual discipline in which the microscopic appearances of lesions are of paramount diagnostic importance. However, the anatomical pathologist cannot by means of morphological features alone distinguish the relatively benign-behaving FAP adenoma from an aggressive Lynch syndrome adenoma. A far more important diagnostic clue for differentiating FAP and Lynch syndrome is the number of polyps.

Apart from the intrinsic limitations of morphologically-based classifications, it may be argued that detailed descriptions of the classical microscopic features of colorectal polyps and cancers are not only of limited educational value to the non-pathologist but contribute relatively little to the global appreciation of the distinguishing phenotypes of the various forms hereditary colorectal cancer. To be sure, one can describe the macroscopic and microscopic appearances of an adenoma and then equate the finding of at least 100 colorectal adenomas with FAP. However, once one moves away from classical FAP to consider other forms of polyposis and hereditary colorectal cancer, it becomes increasingly difficult to generate a description of phenotype that is accurate, comprehensible to the non-expert, and fits with a specific gene disorder. While such descriptions do feature in standard texts and articles [7], the “classical” accounts frequently and frustratingly do not match with what one observes in an individual patient.

In part for the reasons outlined above, the gold standard for the diagnosis of a genetic disorder is the demonstration of a pathogenic mutation that disrupts the function of a gene that has been linked causatively with the condition in question. Nevertheless, phenotypic descriptions facilitate the initial clinical diagnosis of a known genetic disorder and may even lead to the recognition of new disorders. Detailed investigation of genotype–phenotype correlation depends upon the

meticulous attention to phenotypic variation. These descriptions will be generated in diagnostic reports that may relate either to endoscopically biopsied polyps or to lesions in surgical specimens. The information in these reports may subsequently be made available to basic scientists as well as the clinicians (surgeons, gastroenterologists, oncologists or geneticists) and genetic counselors caring for the patient. Nevertheless, and for a variety of reasons that will be outlined below, these reports may be lacking in detail and even accuracy.

9.2 Rarity and Phenotypic Complexity of Hereditary Colorectal Cancer Syndromes

The discipline of pathology, like all disciplines, is learned through practice. Hereditary colorectal cancer syndromes are rare and pathologists have few opportunities to refine their skills. Textbooks are of limited help because they tend to focus on a macroscopic and microscopic description of classical cases rather than on the problems of laboratory work-up and differential diagnosis [7]. The classification of polyposes has traditionally been based on the classification of the individual types of polyp, for example:

Adenoma	Familial adenomatous polyposis
Hyperplastic polyp	Hyperplastic polyposis
Peutz–Jeghers type hamartoma	Peutz–Jeghers syndrome
Juvenile polyp	Juvenile polyposis

Outside FAP and Peutz–Jeghers syndrome, however, one frequently finds forms of polyposis in which the polyps do not fit the description of the classical histological types and/or different types of polyp occur within the same surgical specimen. The pathologist needs to be particularly aware of the less “conventional” forms of polyposis to ensure that sufficient numbers of lesions are identified and sampled. However, the achievement of a working diagnosis will require more than the careful examination of a surgical specimen.

Polyps may have been removed or biopsied endoscopically prior to surgical resection and the resulting information should guide the approach to the surgical specimen and developing the final diagnosis. If the initial endoscopic work-up was undertaken in a different institution, the clinician should bring this to the pathologist’s notice. The pathologist will often be provided with no clinical information beyond age and gender. Apart from the previous polyp history, clinicopathological correlation will be assisted by details of personal history, including extra-colonic lesions, and family history. Finally, the traditional emphasis on polyp type as the basis for the classification of polyposes has eclipsed other and perhaps equally basic polyp attributes such as size, appearance, anatomic location, and the number of polyps. A consideration of these latter features is pivotal to the understanding of phenotypic diversity and ultimately to diagnosis and management.

9.3 Labeling a Mucosal Lesion as a Polyp: The Problem of Size

A polyp is a circumscribed lesion that projects above an epithelial surface. A polyp may be either pedunculated with a stalk of varying length (the archetypal polypoid form) or may be broad-based or sessile. The stalk is generally covered by normal epithelium. The term "polyp" is therefore a gross description that gives no indication of the underlying tissue change or histogenesis. Most polyps are benign lesions but colorectal cancers can be polypoid. Following the demonstration and subsequent acceptance of the adenoma–carcinoma sequence [8, 9], the adenoma rapidly assumed pride of place as the most important type of colorectal polyp. It was the only type of epithelial polyp considered to be neoplastic and the only type that was regarded as having malignant potential. These assumptions will be challenged in a later section on terminology and polyp classification. The aim of this section is to explore the fact that some adenomas are not polypoid, either because they are very small, or flat, or both.

Animal models of colorectal tumorigenesis introduced the concept of minute and non-polypoid lesions with malignant potential. Following the administration of carcinogens, such "aberrant crypt foci" were visualized by staining the surface of the colonic epithelium with a dye such as methylene blue [10, 11]. Under the dissecting microscope, the clusters of aberrant crypt openings were recognized by their increased size and increased staining intensity. Using a similar technique, similar appearing minute lesions were subsequently identified in human colonic mucosa [12, 13]. However, histological examination showed that these were frequently the minute counterparts of the two commonest types of colorectal polyp: adenoma and hyperplastic polyp. In FAP specimens, virtually all such lesions are micro-adenomas. However, in the colorectum of non-FAP patients, most of these lesions are either micro-hyperplastic polyps with serrated crypts or comprise clusters of slightly widened crypts with tufting of the surface epithelium but minimal epithelial serration [12, 13]. Interestingly, the serrated variant is associated principally with *BRAF* mutation while *KRAS* mutation is more common in non-serrated lesions [14]. Outside FAP probably no more than 5% of these minute lesions are micro-adenomas [15]. Like the term "polyp," "aberrant crypt focus" without further qualification confers little meaning in the context of human tissues.

The fact that bi-allelic changes in the *APC* gene are regarded as sufficient for both adenoma initiation and subsequent growth [16] means that time is the only factor that distinguishes a micro-adenoma from a macroscopically visible adenoma. Although micro-adenomas (or oligo-cryptal adenomas) and macroscopically visible adenomas may be biologically and genetically identical, it is impractical to identify and count micro-adenomas as though they were adenomas in evolution. When counting the number of adenomas in polyposis specimens (see below), only lesions measuring 2 mm or greater should be included. Below this size, it is difficult to distinguish actual lesions from insignificant mucosal irregularities or small lymphoid polyps. The microscopic recognition of micro-adenomas in polyposis

specimens is facilitated by embedding the bowel wall flat (instead of on its side) and preparing sections in the same plane as the mucosal lining. Clusters of transversely sectioned adenomatous crypts or even single crypts (uni-cryptal adenoma) may then be visualized. This approach may be useful for identifying micro-adenomas in subjects with attenuated FAP (see below).

Why should a 2 mm adenoma be visible as tiny nodular excrescence? This question links up with the interesting controversy regarding the "bottom-up" or "top-down" origin of adenomas [17, 18]. One reason for confusion regarding "bottom-up" versus "top-down" is that these terms have been applied to two different (though related) scenarios. The terms have been applied first to the mechanism of initiation of the uni-cryptal adenoma [17, 18] and second to the location of the proliferative zone in established adenomas [19]. The condition FAP provides a unique opportunity for studying the initiation of the uni-cryptal adenoma. The pioneering work of Nakamura and Kino established the "bottom-up" mechanism at the point of initiation [20]. Their micro-reconstruction studies showed that the uni-cryptal adenoma begins as a minute bud or outgrowth close to the base of a normal-appearing crypt. Subsequently, the bud migrates upward in the company of the normal crypt epithelium and at the same time extends into the surrounding lamina propria as a dysplastic or adenomatous tubule. Finally, the opening of the dysplastic tubule is relocated to the surface epithelium from which the uni-cryptal adenoma is suspended. The adenomatous crypt so formed is usually considerably shorter than a normal crypt but undergoes more frequent fission. Through repeated crypt fission or branching, the superficial mucosal compartment is progressively populated by multiple adenomatous crypts (Fig. 9.1). This results in a mass expansion that generates a macroscopically visible small nodule. The adenomatous cells may migrate laterally within the surface epithelium and even down adjacent normal crypts. This downward growth often telescopes or intussuscepts within the normal crypt (snow-plough effect). Therefore, even if the initiation of the neoplastic process is "bottom-up," "top-down" growth

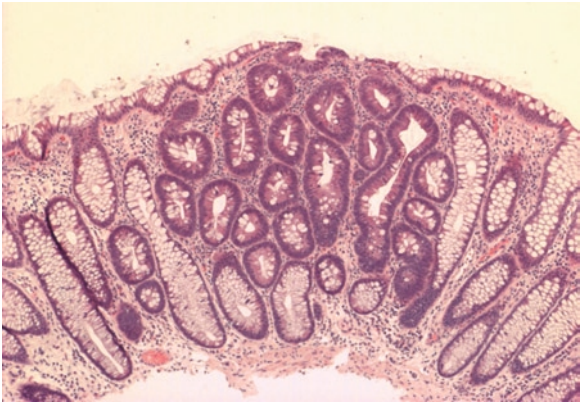

Fig. 9.1 Micro-adenoma from patient with familial adenomatous polyposis. Haematoxylin and eosin

will occur subsequently [18]. Additionally, the fact that proliferating adenomatous epithelium occupies the superficial compartment of the polyp while residual normal crypts dominate in the lower mucosal compartment has also invited the use of the term "top-down" [19].

Not all adenomas develop a morphology in which the proliferative zone is located within the most superficial compartment of the polyp. In villous adenomas, serrated adenomas, and some tubular adenomas, the dysplastic epithelium occupies both superficial and deep compartments and residual normal crypts are inconspicuous [19]. Importantly, proliferation is maximal in the deeper compartments (hence "bottom-up") while maturation with loss of proliferative capacity occurs as the cells migrate toward the epithelial surface. In other words, some adenomas retain the base to surface gradient of differentiation that is seen in normal mucosa (and in hyperplastic polyps). This very striking but under-appreciated difference in the tissue patterning of different classes of adenoma is arguably the most apt justification for the use of the terms "bottom-up" and "top-down."

9.4 Labeling a Mucosal Lesion as a Polyp: The Problem of Flatness

The preceding account leads seamlessly to the explanation of flatness. Adenomas composed of parallel tubules with basal zone proliferation and "bottom-up" maturation (recapitulating normal mucosa) are likely to spread laterally (through crypt division) and to remain flat. Should there be crypt elongation and/or villous change then the adenoma will become more protuberant but may remain sessile. The explanation for adenomas becoming pedunculated is often rather banal. With increasing size, the adenomatous mass may be propelled by peristaltic forces leading to the development of a stalk covered by normal mucosa. This is much more likely to occur in segments of the colon where there is mucosal redundancy and prolapse, notably the sigmoid colon. This explains the tendency for proximal adenomas to be flat or sessile and distal adenomas to be pedunculated [21].

There is evidence that the genetic pathways underlying flat adenomas may differ from those of polypoid adenomas. There is a lower frequency of *APC* and *KRAS* mutation in flat adenomas and multiple differences have been highlighted through gene expression profiling [22–24]. Large and/or villous adenomas are more likely to have *KRAS* mutation [25] and are also more likely to be protuberant or polypoid. The clinical importance of flatness is that the underlying lesion will be more difficult to recognize by both endoscopist and pathologist, malignant invasion will directly involve the underlying submucosa without having to pass first through the altered tissues of the head, neck, and stalk of a polypoid adenoma [26], and flat adenomas may be intrinsically more aggressive [27]. If flat adenomas occur more frequently in Lynch syndrome, then this may simply reflect the fact that the adenomas in the condition are more likely to be right-sided [3].

9.5 Assessing Polyp Numbers

One generally counts objects only when one knows the nature of the objects in question. Of the various types of colorectal polyp, the adenoma is the type that is most likely to be counted. This deceptively simple exercise will be considered in the context of FAP since the vast majority of polyps presenting in this condition are adenomas. Furthermore, the assessment of polyp numbers serves as the basis for distinguishing patients with multiple adenomas from patients with FAP. Colorectal adenomas occurring sporadically (singly or in small numbers) may be chance events while their occurrence in prodigious numbers implies an underlying genetic predisposition. Using the model provided by FAP, it has been shown that a single molecular event, namely disruption of the *APC* gene, is responsible for both the initiation and the subsequent growth of the adenoma [16]. At this point, it is instructive to consider the molecular mechanisms underlying adenoma growth in more detail.

Loss of the APC protein prevents the normal degradation of the transcriptional co-activator β-catenin, and this in turn sends the Wnt signaling pathway into overdrive [28]. Nevertheless, in order for an adenoma to be initiated and then to grow into a recognizable lesion there must be an optimal level of signaling mediated by β-catenin. This depends on a certain level of residual APC function as opposed to the complete loss of APC protein [29, 30]. The APC protein includes a β-catenin regulating domain that comprises seven amino acid repeats (20 amino acids in each repeat). At the gene level, there will be a total of 14 such repeats in each normal cell (since each cell contains two copies of the gene). Most germline mutations (first hit) causing classical FAP leave the mutant gene with only a single repeat and therefore a total of eight repeats within each cell. This is adequate for normal cell function. The usual second hit is caused by mitotic recombination with loss of the wild type allele (loss of seven repeats) and duplication of the mutant germline allele. This will leave a total of only two repeats in the cell. This appears to be the optimum dose for the initiation and subsequent growth of an adenoma. In the situation where the germline mutation causes complete loss of APC function, the second or somatic hit is not associated with loss of heterozygosity but is typically a mutation causing loss of five repeats. This will again leave a total of two repeats in the cell [29, 30]. This has been referred to as the "just right" signaling model in which specific *APC* alterations are selected on the basis that a particular level of residual APC function is required to optimally drive the Wnt-signaling cascade and in turn tumorigenesis [30].

Attenuated FAP (AFAP) is characterised by relatively small numbers of adenomas. Indeed the first family to be recognized with this condition was initially diagnosed as a Lynch syndrome family [31]. The fact that the adenomas were proximal and flat in this family planted the idea that adenomas in Lynch syndrome are typically flat as well as proximally located. Further work on this and other families showed that adenomas in AFAP typically numbered less than 100 and that the age of presentation of adenomas and cancer was older than in classical FAP [32]. Additionally, AFAP was shown to be associated with specific germline mutations

in *APC*: the 5′ end (codons 1–177, exons 1–4), the 3′ end (beyond codon 1580), and the alternatively spliced region of exon 9 (codons 311–408) [33].

It has been suggested that one of the reasons why subjects with AFAP have small numbers of adenomas is because the nature of the germline mutation makes it less likely that a second hit will generate the optimum dose of amino acid repeats. In order to generate the optimum dose for initiating adenomas, a "third hit" within the allele with the germline mutation may be required [34, 35]. This would introduce an important rate limitation and explain the delayed and lower frequency of adenoma initiation. However, one must ask the simple question: do subjects with AFAP always have small numbers of adenomas? In a recent study of 35 subjects with a genetic diagnosis of AFAP, the total polyp count was available for 24 subjects and exceeded 100 in 12 AFAP patients. In fact, seven of the 24 subjects (29%) had 500 polyps or more [33]. The suggestion that AFAP is characterized by fewer than 100 adenomas was challenged in an earlier study of four patients who had a history consistent with classical FAP but had less than 20 adenomas with standard colonoscopy. When colonoscopy was augmented with dye-spray, each of the four subjects was found to have over 1,000 polyps. These adenomas were sufficiently large to be recognized and counted within the subsequent colectomy specimen [36].

The preceding observations suggest that AFAP allelotypes involving only two hits are sufficient for initiating adenomas but that the adenomas then grow relatively slowly, remain small for an extended period, and are consequently overlooked by both the endoscopist and the pathologist. The existence of a third hit in some polyps, and particularly in association with germline mutation of the alternatively spliced region in exon 9, has been clearly demonstrated [33]. However, the third hit may not be necessary for adenoma initiation but may potentiate adenoma growth. Large adenomas may be selected more frequently for genetic analysis and this could inflate the frequency adenomas with a third hit.

In order to understand the mechanisms of adenoma initiation, to derive meaningful phenotype–genotype correlations, as well as to correctly diagnose of FAP, it is clearly important that adenomas should be identified and an estimate made of their numbers. It is impossible to remove and diagnose every polyp in a surgical specimen of adenomatous polyposis. However, a reasonable assessment of polyp numbers can be made by counting polyps within a 10 cm^2 field (roughly the area within a 35 mm transparency or projection slide) from five different regions of the colon (ascending colon, transverse colon, descending colon, sigmoid colon, and rectum), obtaining an estimate of the total mucosal area within each region, and extrapolating the total count accordingly. On the basis of the observations noted above, it seems likely that the number of adenomas is underestimated when they remain very small. Small adenomas may be indistinguishable from hyperplastic polyps grossly and histological diagnosis of a representative subset will therefore be required. AFAP is genetically and phenotypically heterogeneous [33]. However, subsets may in time come to be viewed as variants of FAP in which adenomas are as numerous as in the classical condition but remain relatively small.

9.6 Anatomic Location of Polyps and Cancers

In general, adenomas in FAP have a pan-colorectal distribution, though may be more numerous and/or prominent in the proximal colon in AFAP [32]. Exactly the same distributional pattern may be faithfully recapitulated among different members of the same polyposis family [33], indicating the importance of genotype over environment in this condition. As noted above, adenomas as well as carcinomas are more common in the proximal colon in Lynch syndrome. However, this difference is not dramatic, and indeed, around 40% of CRCs in Lynch syndrome occur in the left colon and rectum.

A subset of sporadic polyps and sporadic CRCs with a marked predilection for the proximal colon is the subset with mutation of the oncogene *BRAF* and extensive DNA methylation [37, 38]. Around 50% of CRCs with these changes will also show DNA microsatellite instability (MSI) due to methylation of the DNA repair gene *MLH1* [39]. CRCs with *BRAF* mutation (almost invariably associated with extensive DNA methylation) cluster within families [39–41] and it has been suggested that there is an underlying genetically determined predisposition to DNA methylation within such families [42]. This predisposition to DNA methylation is already fully developed within the pre-cancerous polyps [43]. The precursor lesions are not adenomas in the conventional sense but are variant hyperplastic polyps that are relatively large and sessile (see below under variant polyps). The typical hyperplastic polyp is found in the rectum and distal colon while variant hyperplastic polyps may occur both singly and multiply and show a proximal predilection [44]. It has been suggested that the presence of at least five hyperplastic polyps proximal to the sigmoid colon is sufficient for a diagnosis of hyperplastic polyposis provided that at least two of the polyps are >10 mm [45]. An alternative definition of hyperplastic polyposis stipulates >30 hyperplastic polyps with a pan-colorectal distribution and no size limitation [45]. Many patients with hyperplastic polyposis will in fact meet both definitions. However, it is the phenotype characterized by large and proximally located variant polyps that is likely to be closely linked with the serrated pathway of colorectal tumorigenesis [46]. Hyperplastic polyposis is usually an isolated finding but the condition can cluster in two or members of the same family, suggesting an autosomal recessive trait [47, 48].

9.7 Classification of Colorectal Polyps

9.7.1 Neoplastic Versus Non-Neoplastic Polyps

Tissues display a relatively limited repertoire of responses to different pathogenic stimuli. Notwithstanding this well-known maxim, it is possible to discern considerably more morphological heterogeneity among colorectal polyps than was appreciated in only the recent past. Traditionally, polyps other than adenomas have been regarded as non-neoplastic overgrowths caused, for example, by an increase in cell numbers (hyperplastic polyps) or abnormal tissue development (hamartomas including

juvenile polyps, Peutz–Jeghers polyps and Cowden-type polyps) [8]. It is interesting that hyperplastic polyps were originally termed metaplastic polyps [49] since, in expressing gastric mucins (MUC5AC) [50] and small intestinal type sialomucin (loss of colonic O-acetyl substituents) [51], these polyps are indeed characterized by metaplasia or trans-differentiation. The underlying disorder in hyperplastic polyps is not a mild over-generation of cells but a failure of programmed cell death or apoptosis. As a consequence, cells are retained beyond their normal lifespan and therefore show features of hypermaturation and senescence [52]. Inhibition of apoptosis is generally understood to be central to the process of neoplasia. Hyperplastic polyps are characterized by clonal genetic alterations. Most have mutation of a cancer-associated proto-oncogene (either *KRAS* or *BRAF*) [37, 53], many show methylation of a variety of loci including the promoter regions of tumor suppressor genes [53, 54], and a subset shows microsatellite instability [55]. As a disorder of growth and differentiation with cancer-associated clonal genetic alterations, the hyperplastic polyp fully meets the defining criteria of a neoplasm [56]. It is true that the vast majority of hyperplastic polyps will not become malignant, but this is also true of colorectal adenomas.

9.7.2 Variant Polyps

It was primarily through the study of polyposis syndromes that the link between "non-neoplastic" polyps and CRC first became apparent. Within the polyposes, the macroscopic and microscopic appearances of polyps do not always conform to the traditional descriptions of their sporadic counterparts. In juvenile polyposis, for example, variant polyps are characterized by large size, multi-lobation, and an increased epithelial–stromal ratio [57] (Fig. 9.2). In hyperplastic polyposis, variant polyps remain sessile but are relatively large and show a variety of distinguishing features with respect to architecture (exaggerated serration and crypt dilatation), differentiation (hyper-mucinous epithelium), and proliferation [46]. It was suggested that hyperplastic polyposis should be renamed "serrated adenomatous polyposis" [46]. Although this recommendation has not been widely adopted, the same variant polyps have been shown to occur sporadically and have been renamed as "sessile serrated adenomas" (SSA) [44]. The SSA lacks the overtly dysplastic cytology of the usual type of colorectal adenoma. The term adenoma is used to indicate that these lesions are epithelial neoplasms with malignant potential and can be distinguished from typical hyperplastic polyps on histological grounds [44].

9.7.3 Mixed Polyps

Conceptually even more challenging than these variant polyps are polyps with "mixed" features. It should be noted that the term "mixed" has been used in two ways when applied to an individual polyp. First, it has been used to indicate a composite appearance such that the polyp may appear to have formed through the collision of two different types of polyp (for example, a hyperplastic polyp

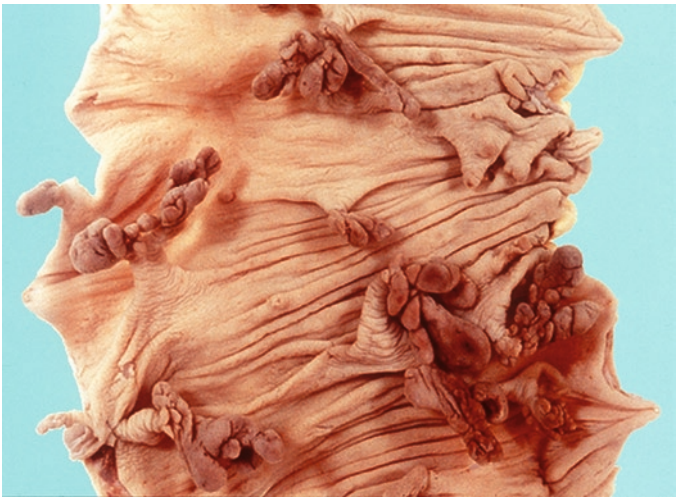

Fig. 9.2 Multi-lobated colorectal polyps from a subject with juvenile polyposis

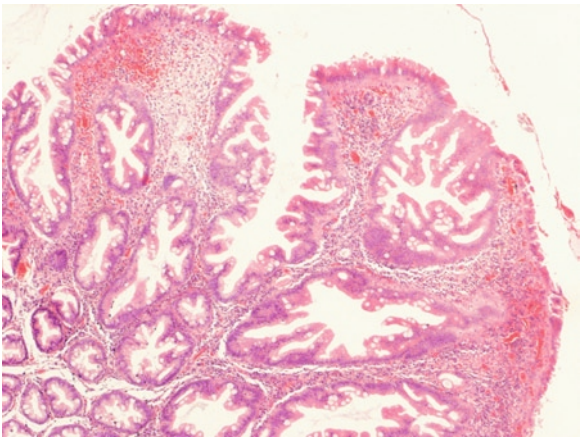

Fig. 9.3 "Mixed" or "blended" polyp from a subject with hereditary mixed polyposis syndrome. The polyp has the spherical contour (smooth epithelial surface) and expanded lamina propria of a juvenile polyp, the serrated architecture of a hyperplastic polyp, and the cytological dysplasia of an adenoma. Haematoxylin and eosin

and an adenoma) [58]. Second, the term mixed has been used to describe a polyp with a "blended" appearance. Such a polyp has the same microscopic appearance throughout, but the component features may recall more than one type of polyp. For example, the lamina propria may be expanded as in juvenile polyp, the crypts may be serrated as in a hyperplastic polyp (or SSA), while the crypt epithelium may show the overtly dysplastic cytology of an adenoma (Fig. 9.3). Polyps composed of serrated crypts but with an overtly adenomatous appearance were initially described as mixed hyperplastic adenomatous polyps [59] but the term "serrated adenoma" is now preferred [58, 60]. To avoid confusion with SSA,

these overtly dysplastic serrated polyps have been termed "traditional serrated adenoma" (TSA) [60].

Currently, therefore, the term mixed polyp is generally used only in the first sense to describe a polyp with two (or more) discrete components. However, it is likely that most, if not all, mixed polyps are not chance collisions of unrelated polyps but represent the transformation of less advanced to a more advanced lesion. This conclusion fits with the demonstration of identical mutations and shared histological features, such as crypt serration, within both components of a mixed polyp [55].

9.7.4 Serrated Polyps

The preceding observations led directly to the concept of a "serrated pathway" of colorectal tumorignesis encompassing all serrated lesions from the micro-hyperplastic polyp ("aberrant crypt focus") through to advanced serrated polyps (SSA, TSA and mixed polyps) [16, 61]. The term "serrated" refers to the saw-tooth, scalloped, or corrugated contours of crypts. In normal colorectal mucosa and in most tubular adenomas, crypts have a smooth or test-tube-like contour. Serration occurs through a variety of mechanisms: (1) the presence of increased cell numbers results in simple buckling of the epithelium, (2) the cells lining the crypts vary in height with tall columnar cells protruding into the crypt lumen as "hills" and shorter goblet cells forming intervening "valleys," (3) the development of deeper valleys caused by the formation of intra-epithelial micro-crypts along the parent crypt epithelium (Fig. 9.4), and (4) the out-pouching of enlarging micro-crypts as multiple small glands surrounding the parent gland [62]. Not only are there multiple ways of producing a serrated contour but serration can be observed to occur as a minor or secondary component in juvenile polyps,

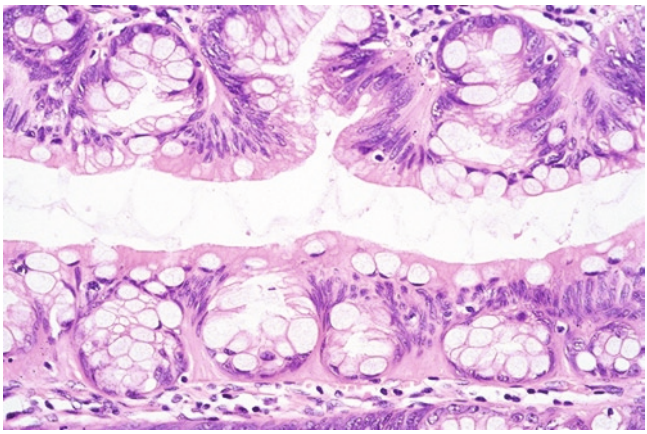

Fig. 9.4 Adenomatous (dysplastic) epithelium with a serrated contour produced by the development of numerous intra-epithelial micro-glands. This is the basis for the serrated appearance of the traditional serrated adenoma. Haematoxylin and eosin

inflammatory polyps, polyps caused by mucosal prolapse, and conventional adenomas. However, intra-epithelial micro-crypts are the hallmark feature of TSAs [62]. Despite the fact that crypt serration is not restricted to a single type of colorectal lesion, the recognition of a serrated pathway of colorectal tumorigenesis heralded the fact that CRC must be regarded as a *multi-pathway disease* [63, 64].

9.8 Types of Colorectal Cancer

The serrated pathway to CRC is characterized by genetic alterations, for example, mutation of *BRAF*, extensive DNA methylation, and DNA microsatellite instability (MSI), which are not seen in sporadic CRCs that develop through the classic adenoma–carcinoma sequence [63]. The latter are characterized by mutation of *APC*, *KRAS,* and *TP53* and chromosomal instability [65]. Although polyps and CRCs may be grouped according to molecular pathways showing little or no overlap, some polyps and CRCs may combine elements of the independent pathways into "fusion" pathways [66]. CRCs in Lynch syndrome are a particularly good example of such a "fusion." Genetic alterations shared with the serrated pathway include MSI, lack of chromosomal instability, and mutation of particular tumor suppressor genes that have repetitive coding sequences (*TGFβRII*, *IGF2R* and *BAX*). On the other hand, CRCs in Lynch syndrome lack both mutation of *BRAF* and extensive DNA methylation, develop in adenomas, and show frequent mutation of *APC* and/or *KRAS* [67, 68].

The genetic heterogeneity of CRC is matched by morphologic heterogeneity to the extent that the vast majority of CRCs with MSI can be recognized on the basis of particular microscopic features [69]. These features include poor differentiation, mucinous differentiation, tumor-infiltrating lymphocytes (Fig. 9.5), and a Crohn-like

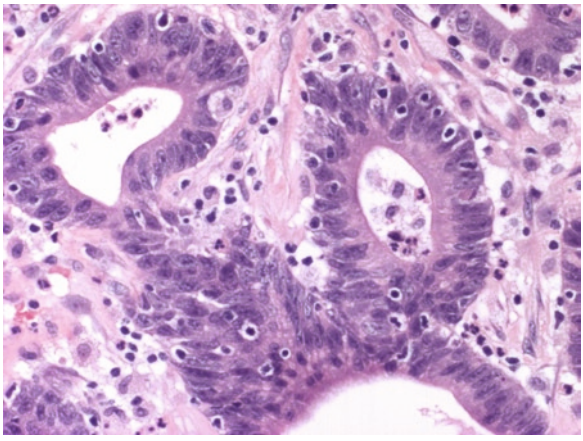

Fig. 9.5 Tumor-infiltrating or intra-epithelial lymphocytes in a colorectal cancer from a subject with Lynch syndrome. Haematoxylin and eosin

lymphocytic reaction [69–72]. The link between morphology and MSI applies to CRCs presenting below the age of 60 years [73]. Many such early-onset CRCs with MSI would be expected to occur in the context of Lynch syndrome. The pathologist is therefore able to identify CRCs that should be worked up to confirm or exclude this possibility. Immuno-histochemical studies not only serve to indicate a deficiency of DNA mismatch repair but point to the likely underlying germline defect in *MLH1*, *MSH2*, *MSH6,* or *PMS2* [74]. Nevertheless, the contribution by the pathologist to the diagnosis of Lynch syndrome needs to occur within the context of a multidisciplinary cancer genetics service.

9.9 Comprehensive Description of Phenotype: A Difficult Task

It is a relatively simple matter to consider polyp size, gross appearance, number, location, and type in isolation. A greater level of complexity is introduced when time is factored in. First the number, size, and developmental stage of the polyp will be dependant upon the age of the patient. Second, the number of endoscopically detected polyps will accumulate over time. Finally there is the histological complexity afforded by the existence of the variant and mixed types of polyp. The integration of this information into a comprehensive phenotype (or at least one that is optimally informative) is difficult in the extreme. This is because it is often impossible to force mixed polyps into the existing rigid and simplistic classification of colorectal polyps. Additionally, two, three, or more types of polyp may occur within the same patient giving a "mixed polyposis." A "mixed polyposis" phenotype may occur in the context of hereditary mixed polyposis syndrome (linked to chromosome 15q) [75], juvenile polyposis (germline mutation of *SMAD4* or *BMPR1A*) [57], Cowden syndrome (germline mutation of *PTEN*) [76], and hyperplastic polyposis (genetic basis unknown) [77]. Polyps other than adenomas may sometimes occur within the adenomatous polyposes including FAP, attenuated FAP, and *MYH*-associated polyposis [78]. Hyperplastic polyps have sometimes been sufficiently numerous in *MYH*-polyposis to cause confusion with hyperplastic polyposis [48]. As a direct result of this phenotypic complexity, the literature has not escaped from error and has sometimes generated confusion. For example, the *PTEN* gene was originally linked with juvenile polyposis [79], while a family with a germline mutation of *BMPR1A* was labeled as having hereditary mixed polyposis syndrome [80]. In a survey of 49 subjects with unexplained hamartomatous and hyperplastic polyposis, two subjects with "hyperplastic polyposis" had germline mutation of *PTEN* that is linked to Cowden syndrome [81]. The colorectal polyps in Cowden syndrome have been described as hamartomatous, inflammatory, and occasionally adenomatous [76]. It is conceivable that some Cowden polyps could develop glandular serration, but a true likeness to hyperplastic polyposis seems implausible. Scattered ganglion cells may occur in the relatively dense stroma of a Cowden polyp [7] (Fig. 9.6). Gangioneuromatous features have

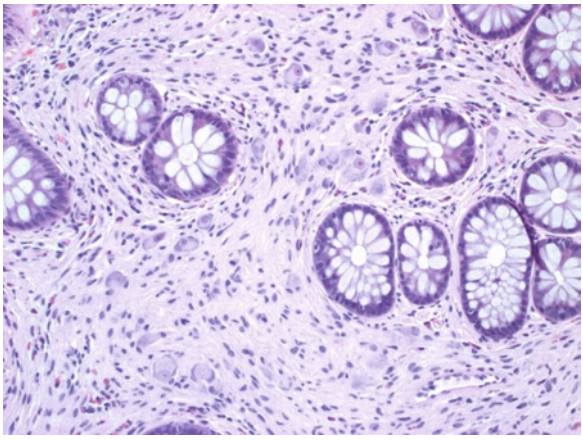

Fig. 9.6 Scattered ganglion cells in the stroma of a Cowden syndrome hamartomatous polyp. Haematoxylin and eosin

also been described in juvenile polyposis [82, 83]. In this author's view, ganglion cells are rarely associated with juvenile polyposis [57], but are in fact a frequent and diagnostically useful finding in Cowden polyps.

To some extent, these problems of misdiagnosis could be avoided by a greater and more direct input by pathologists into the literature. If pathologists are included in the authorship of articles at all, it is often because they merely provided some tissue samples. Yet, even with the full involvement of an expert pathologist in the re-review cases [81], establishing a link between phenotype and genotype may not be simple. Phenotypic variability may be caused by commonly occurring polymorphisms or even by the involvement of two of the known syndromic genes within the same family. For example, large deletions in 10q implicating both *BMPR1A* and *PTEN* have been demonstrated in infants with a severe form of juvenile polyposis [84]. On the basis of all the preceding problems relating to polyp size, gross appearance, number, location, type, variant types, mixed types, and the frequent phenomenon of "mixed polyposes," it is helpful to develop a checklist of guidelines for a comprehensive description of phenotype.

9.10 Checklist for Description of Phenotype

1. Not all polyps can be sampled, but sampling should be representative with respect to size, appearance, and anatomic location.
2. In the presence of multiple large polyps and/or cancers, deliberately count or estimate the number of polyps between 2 and 10 mm and sample at least ten of these small polyps. In terms of actual numbers, the small polyps may dominate and be the clue to the underlying disorder.

3. Give an indication of the dominant polyp type: for example, adenoma, hyperplastic polyp, or juvenile polyp, as this will have a strong bearing on the working diagnosis.
4. The age of the patient will influence the number, size, and developmental stage of polyps.
5. Consider sectioning the mucosa *en face* to identify and classify micro-polyps.
6. Scrutinize the proximal colon for inconspicuous sessile lesions. Hyperplastic polyps and sessile serrated adenomas may be small, pale, present in relatively small numbers, and easily overlooked.
7. Describe mixed types of polyp that do not fit readily with any standard type.
8. Hamartomatous polyps with a relatively dense fibroblastic stroma, crypts clustering in a lobular arrangement, and stromal ganglion cells are suggestive of Cowden syndrome and not juvenile polyposis.
9. Hereditary mixed polyposis syndrome (linked to 15q) has been described in only four families (Ashkenazi Jewish) and one should therefore be cautious in suggesting such a rare diagnosis.
10. Attempt to provide a cumulative description when polyps have been removed on multiple occasions.
11. Since serrated polyps can be conceived as a continuum, count all serrated polyps (hyperplastic polyps, variant serrated polyps, mixed polyps and serrated adenomas) when evaluating a possible case of hyperplastic polyposis.
12. Obtain relevant personal and family history whenever possible.
13. Utilize information with respect to extra-colonic pathology when suggesting a working diagnosis.
14. Assess features associated with MSI in CRCs presenting below age of 60 years.

In summary, this review has provided a systematic analysis of the considerable challenges associated with generating a comprehensive description of the phenotype of hereditary forms of colorectal cancer and/or colorectal polyposes. Nevertheless, the proffered advice should allow the non-expert to negotiate the difficulties and thereby assist in achieving the correct genetic diagnosis as well as furthering our understanding of these rare but important disorders.

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Part IV
Clinical Science: Familial Adenomatous Polyposis

Chapter 10
Genotype Phenotype Correlation in Familial Adenomatous Polyposis

Miguel A. Rodriguez-Bigas

Abstract Familial Adenomatous Polyposis (FAP) is an autosomal dominant inherited syndrome whereby affected individuals can have less than 100 adenomas (Attenuated Adenomatous Polyposis) to thousands of adenomas (Classic Familial Adenomatous Polyposis). The syndrome is also characterized by extracolonic manifestations such as fundic gland polyps, duodenal adenomas, desmoids tumors, thyroid cancer, osteomas, and congenital hypertrophied pigmented retinal epithelium among others. There are genotype and phenotype correlations noted in and these will be discussed in this chapter.

Keywords Familial adenomatous polyposis • Attenuated familial adenomatous polyposis • Genotype/phenotype • Mutation cluster region • Extracolonic manifestations • Rectal cancer

Classic familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome characterized by the presence of hundreds to thousands of adenomas throughout the large bowel. The penetrance in FAP is close to 100%. The majority of germline mutations in the APC (Adenomatous Polyposis Coli) gene are truncating mutations. The phenotypic manifestations in FAP are not limited to the large bowel. As noted in previous chapters, there are extracolonic manifestations in FAP such as duodenal adenomas, fundic gland polyps, gastric adenomas, desmoids tumors, congenital hypertrophy retinal pigmented epithelium (CHRPE), thyroid cancer, osteomas, and others. Not all the patients will express these manifestations. Even in the large bowel, there is bound to be intra and interfamilial variation in individuals with the same mutations, thus suggesting that other factors, such as the environment or modified genes, could come into play in terms of phenotypic manifestations of the syndrome. In fact, a milder form of the syndrome Attenuated

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Table 10.1 Overview of genotype–phenotype correlations in familial adenomatous polyposis

Familial adenomatous polyposis		
Genotype/phenotype		
Phenotype	Genotype	References
Profuse polyposis	Codons 1250–1464	Nagase, Caspari, Bertario, Friedl [11, 14, 15, 27]
Attenuated polyposis	Exons 4.5 Exon 9 Exon 15(3′ end)	Spirio, Soravia [6, 17]
CHRPE	Exons 9–15	Olschwang [32]
Osteomas	Codons 757–1513	Bisgaard, Bulow [22, 33]
Desmoids	Codons 1445–1578	Caspari, Bertario [14, 29]
Thyroid cancer	5′ codon 1220	Cetta [34]
Rectal cancer risk after IRS	Codons 1250, 1309, 1328, 1250–1464	Vasen, Wu, Bertario [14, 25, 26]

Adenomatous Familial Polyposis (AFAP), where adenomas develop later in life and are predominantly right sided, has been described. In this chapter, we will address the genotype–phenotype correlations in FAP.

In classic FAP, in the era of endoscopic surveillance, the median age at diagnosis of adenoma is approximately 15 years. [1]. In untreated patients, the mean age of colorectal cancer diagnosis and colorectal cancer death is 39 years and 42 years, respectively [2]. In AFAP, colorectal cancer will generally occur in the early 50s [3–5]. The syndrome is caused by mutations in the adenomatous polyposis gene (APC), a gene with 2,843 amino acids in 15 coding exons located in the long arm of chromosome 5 [6, 7]. There are more than 800 germline APC mutations reported to the APC mutation database [8]. There have been clear associations between the genotype and the phenotype in FAP patients (Table 10.1). However, from the table it can be seen that there is overlap in the genotype and phenotype in these patients in terms of colonic and extracolonic manifestations.

However, the second (somatic) mutation apparently depends on the site of the first mutation [9]. Depending on the location of the first hit, the second hit may be either an allelic loss or another truncating mutation [10]. The type of second hit mutation may have a bearing on the genotype–phenotype variation in FAP patients. This has been discussed in more detail in Chap. 3 by Carvajal-Carmona et al. [7].

10.1 Colonic Polyposis

Nagase reported that patients with mutations between codons 1250 and 1464 developed profuse polyposis with thousands (>5,000) of colorectal adenomas [11]. Other investigators have also reported that individuals with mutations between codons 1250 and 1311 in general develop thousands of adenomas [12, 13]. It is within this region of the APC gene that the most common mutation associated with FAP occurs. The mutation at codon 1309 in exon 15 has been associated with severe polyposis (thousands of polyps), and early age onset of colorectal cancer [14, 15].

An intermediate as well as an attenuated phenotype in FAP have been described. In the intermediate polyposis phenotype associated with mutations between codon 157 and 1595 (excluding the mutation cluster region 1250–1264), patients develop hundreds to thousands of adenomas [11]. In the attenuated phenotype, patients usually develop less than 100 adenomas which are mainly located in the right side of the colon. Mutations in the APC gene have been reported at the extreme ends of the gene in exons 3, 4, 5, and 15 as well as in exon 9 [6, 16–19]. Others have reported that mutations in these regions can lead to highly variable phenotypes including the attenuated, intermediate, and profuse [4, 20]. The reader is referred to a review by Nieuwenhuis and Vasen where detailed information of these and other genotype–phenotype correlations are given [21].

10.2 Rectal Cancer Risk

Rectal cancer remains a significant cause of mortality in patients with FAP after prophylactic abdominal colectomy and ileorectal anastomosis (IRA). The incidence of rectal cancer has been reported between 15% and 40% after prophylactic colectomy. These widespread percentage differences may have been related to the length of the remaining rectum and to the fact that even patients with thousands of polyps throughout the colon and rectum underwent an abdominal colectomy and an ileorectal anastomosis as it was the only alternative to proctocolectomy and ileostomy. In these older series, the endoscopic surveillance could have also influenced the development of rectal cancer. Church and Bulow have demonstrated in more recent studies that in part with better patient selection as well as better endoscopic surveillance the risk rectal cancer has decreased to 10% or less after abdominal colectomy in the era of ileal pouch anal anastomosis [22, 23]. Debinski et al. have reported that polyp count influences the risk of cancer in FAP patients [24]. Moreover, there have been three studies reporting the genotype–phenotype correlation in patients who developed rectal cancer after abdominal colectomy [14, 25, 26]. Vasen reported that patients with mutation 3′ to codon 1250 had a higher risk of rectal cancer. In the study from the Cleveland Clinic, Wu et al., reported that the prospect of retaining the rectum in patients with mutations at codons 1309 and 1328 was poor. Bertario et al. reported a higher rectal cancer risk in patients with mutations in codons 1250–1464. Therefore, the site of the APC mutation may be one of the factors to consider in terms of the type of prophylactic surgery that will be recommended to patients with FAP.

10.3 Upper Gastrointestinal Polyps

A clear genotype–phenotype correlation with upper gastrointestinal polyps has not been reported in FAP patients. There have been different mutations reported in patients with Upper Gastrointestinal (UGI) polyps including codons 564–1465,

downstream 3′ end of codon 1395, and in exon 4 [12, 17, 21]. There has been no correlation between the genotype and the severity of UGI polyposis [21, 27, 28].

10.4 Desmoid Tumors

Desmoid tumors are a source of major morbidity in FAP. Even though these tumors are not considered malignant, they grow and can invade adjacent organs and structures and can eventually cause the demise of the patients. Desmoids tumors have been generally associated with mutations downstream codon 1444 [14, 21, 29, 30]. This is another factor to consider, especially when considering the timing of prophylactic colectomy. A family with desmoid tumors and no colonic manifestation of FAP with a truncating frameshift mutation at codon 1924 has been described. [31].

10.5 Congenital Hypertrophied Pigmented Retinal Epithelium

In 1993, Olschwang et al. were the first to report that Congenital Hypertrophied Pigmented Retinal Epithelium (CHRPE) almost never occurred if the mutation was 5′ to exon 9 [32]. Subsequently other genotype–phenotype correlations have been reported [21]. The significance of CHRPE is not known. However, in at-risk individuals, where a mutation has not been found in the family, this phenotypic manifestation may serve as a marker of carrier status in the absence of polyposis.

10.6 Osteomas

Similar to CHRPE, osteomas have served as a marker of potential mutation carrier status in at-risk individuals who have not been genetically tested or where a mutation has not been found in the family. In patients from the Danish Polyposis Registry, Bisgard and Bulow reported that osteomas were present only in those patients with mutations between codon 767 and codon 1513 [33].

10.7 Thyroid Cancer

There is not much reported in the literature regarding genotype–phenotype correlations in FAP. However, in a cooperative study and a review of the literature, Cetta et al., reported that the majority of the mutations in FAP patients with thyroid cancer occur in the 5′ end of exon 15 [34]. Specifically 22 of 24 patients had mutations 5′ to codon 1220 (codons 1286–1513).

10.8 Summary

In summary, there are genotype–phenotype correlations in FAP. Important issues dealing with these correlations may include the type of prophylactic surgery (profuse polyposis and rectal cancer risk), the timing of prophylactic surgery (desmoids tumors), and surveillance (thyroid cancer). However, as in any other field in medicine, the most important aspects in the management of these patients are the history and physical examination as well as the patient's own personal considerations and specific circumstances.

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Chapter 11
Surgery for Familial Adenomatous Polyposis

James Church

Abstract Patients with familial adenomatous polyposis are at high risk of developing colorectal cancer. Successful prevention of cancer depends on timely and appropriate surgery. Decisions regarding the timing and choice of surgery are driven primarily by the severity of the polyposis and the social situation of the patient. Immediate surgery is recommended for patients presenting with cancer, while symptomatic patients and those with profuse polyposis should be operated without delay. Asymptomatic patients and those with mild or attenuated polyposis may be operated electively, and surgery can be delayed for years as long as regular colonoscopy shows no increase in cancer risk.

A secondary aim of prophylactic colectomy in familial adenomatous polyposis is preservation of bowel function and quality of life. To this end, patients with mild polyposis are recommended to have a colectomy and ileorectal anastomosis, often performed with minimally invasive technique. Careful selection results in a low incidence of rectal cancer and proctectomy. Severely affected patients have proctocolectomy and ileal pouch-anal anastomosis, and most have acceptable functional outcomes. Lifetime surveillance of residual gastrointestinal tract is mandatory for all patients.

Desmoid disease occurs in 30% of familial adenomatous polyposis patients, and is the second most common cause of death. Patients at high risk of desmoid disease should have the surgical strategy re-evaluated with a view to minimizing the incidence and impact of desmoids.

Keywords Polyposis severity • Timing of surgery • Ileal pouch anal anastomosis • Ileorectal anastomosis • Laparoscopy • Quality of life • Desmoid disease

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11.1 Introduction

Most patients with familial adenomatous polyposis (FAP) need prophylactic colectomy or proctocolectomy to minimize the risk of developing colon or rectal cancer. Decision making regarding surgery therefore revolves more around which operation and when to do it than whether surgery should be done at all. This chapter will discuss the factors that enter in to the decision-making process, and the way that decisions influence outcome.

11.2 Aims of Surgery

Without colectomy or proctocolectomy patients with FAP have a very high risk of developing colorectal cancer. This is obvious from the presentation of the 25% or so of FAP patients who do not have a family history [1]. Because they do not know they have the syndrome they do not present for screening and therefore usually present with symptoms, often rectal bleeding and diarrhea. The incidence of cancer in this group is 60% [2]. The primary aim of surgery therefore is to prevent colorectal cancer by removing adenomatous polyps. The only reason surgery is necessary is because the polyps cannot be reliably controlled endoscopically and because chemoprevention is unlikely to work. The majority of patients with FAP who present for surgery are asymptomatic. In this setting, surgery is truly prophylactic and the second, but almost equally important, aim is to accomplish cancer prevention with minimal sacrifice of quality of life and acceptability of bowel function. This is especially critical for two reasons: one, many FAP patients, especially those without symptoms who were diagnosed on screening, are young and in the middle of their social, educational, and physical development, and two, FAP is a disease of families and to maximize compliance within a family, it is important that each family member has a good experience. Finally FAP cannot be cured by surgery. Polyps will always tend to form in the upper and lower gastrointestinal tract no matter which parts are removed, and tumors also develop in extra-intestinal organs. It is important that patients with FAP receive the right operation at the right time, done with excellent technique, and satisfactory outcomes. It is also important that each patient is part of a program of regular surveillance.

11.3 Factors Influencing Choice and Timing of Surgery

11.3.1 Disease Severity

The concept of severity in FAP relates to the way in which the APC mutation is expressed in various organs. Severity is defined according to the status of the organ in question. For example a “severely” affected duodenum is Spigelman Stage IV,

[3] a "mildly" affected duodenum is Stage I [3]. A "severely" affected rectum has more than 20 synchronous adenomas, or any adenoma with severe dysplasia, or a large adenoma (>3 cm), or a cancer [4]. A "severely" affected colon contains more than 1,000 synchronous adenomas, or a cancer, or a severely dysplastic adenoma, or a lesser number of large (>1.0 cm) adenomas [4]. "Mild" polyposis is the opposite: less than 20 rectal polyps, less than 1,000 colonic polyps, no severe dysplasia. Attenuated polyposis is defined as less than 100 synchronous adenomas and is equivalent in meaning to "oligopolyposis" [5]. Severity of polyposis is important in FAP as it drives the decision regarding the type of surgery. Different definitions are given in Table 11.1.

11.3.2 Desmoid Tumors

Desmoid tumors are histologically benign, but sometimes clinically aggressive growths of fibro-aponeurotic tissue found rarely in the general population, but in up to 30% of patients with FAP [6]. They are dealt with in detail in Chap. 13, but because they impact surgical decision-making they need to be mentioned here. Fifty percent of desmoid tumors develop within the abdomen, after abdominal surgery. They infiltrate the mesentery of the small bowel, puckering the bowel and predisposing to both intestinal and ureteric obstruction. They can restrict the ability of a surgeon to mobilize bowel and therefore may impact surgical strategy (see Table 11.2) [6]. They may cause ischemic bowel due to their effects on mesenteric vessels, producing bowel perforations and fistulas. Mesenteric desmoids in patients with FAP can grow quickly, and are the second most common cause of death, after colorectal cancer [7]. Not all FAP patients are at the same risk of desmoids but for those at high risk it may be better to defer surgery if possible so that desmoids develop later in life when they may be less aggressive, and when their impact on the patient's life is less diastrous. It is also possible that some surgeries are more likely to produce desmoids than others, [8] so that if surgery is inevitable, a less "desmoidogenic" procedure may need to be chosen.

11.4 Surgical Options

Discussion of surgical options involves both the type of procedure and its timing. The majority of patients with FAP will undergo either a colectomy and ileorectal anastomosis (IRA), or a total proctocolectomy and ileal pouch-anal anastomosis

Table 11.1 Severity of adenomatous polyposis

Attenuated	<100 synchronous colorectal adenomas
Classical FAP	>100 synchronous colorectal adenomas
Mild FAP	<1,000 synchronous colorectal adenomas
Severe FAP	>1,000 synchronous colorectal adenomas
Profuse FAP	>5,000 synchronous colorectal adenomas

FAP Familial Adenomatous Polyposis

Table 11.2 Indications, advantages, and disadvantages of IRA and IPAA for FAP

Option	Indications	Contraindications	Advantages	Disadvantages
Ileorectal anastomosis	<20 rectal adenomas, <1,000 colonic adenomas	Severe dysplasia in the rectum, Cancer anywhere in large bowel, Large (>3 cm) rectal adenomas	Avoids pelvic dissection, Simple surgery, Low complications, Good functional result, No stoma	Retained rectum may need to be removed later, Possibility of rectal cancer if patient is not compliant with follow-up
Ileal Pouch-anal anastomosis, stapled	>20 rectal adenomas, >1,000 colonic adenomas, Severe dysplasia in the rectum, Cancer anywhere in large bowel, Large (>3 cm) rectal adenomas, ATZ clear of adenomas	Incompetent sphincters, Rectal cancer invading sphincters, Pouch won't reach anus	Avoids permanent stoma, Good function in most patients	Higher complication rate, May provoke desmoids, Decreased ability to conceive in women. Retained anal and low rectal mucosa may develop neoplasia (28%)
Ileal Pouch-anal anastomosis, mucosectomy	>20 rectal adenomas, >1,000 colonic adenomas, Severe dysplasia in the rectum, Cancer anywhere in large bowel, Large (>3 cm) rectal adenomas, ATZ contains adenomas	Incompetent sphincters, Rectal cancer invading sphincters, Pouch won't reach anus	Avoids permanent stoma, Reasonable function in most patients. No residual anal mucosa (although neoplasia can still occur)	Highest complication rate, May provoke desmoids, Decreased ability to conceive in women, Frequent seepage, Nighttime incontinence. Anal neoplasia in 14%
Proctocolectomy and ileostomy	>20 rectal adenomas, >1,000 colonic adenomas, Severe dysplasia in the rectum, Cancer anywhere in large bowel, Large (>3 cm) rectal denomas, ATZ contains adenomas, Incompetent sphincters, Rectal cancer invading sphincters, Pouch won't reach anus	Competent sphincters, No rectal cancer, Pouch reaches anus	Low complication rate, Low chance of reoperation, No anal incontinence	Permanent stoma

ATZ Anal Transition Zone

(IPAA). Occasionally an ileosigmoid anastomosis is done, and rarely a total proctocolectomy and ileostomy. A permanent ileostomy may be of the continent variety (Kock) or the regular type (Brooke).

11.4.1 IRA

An IRA involves removal of the entire colon, leaving 15 cm of rectum for an anastomosis to the terminal ileum. Leaving less than 10 cm of rectum may predispose the patient to disabling stool frequency. The technical points of a colectomy and IRA are as follows:

Preoperative colonoscopy to assess the risk of a cancer is important. If it is clear that there is no cancer, mesenteric resection may be reasonably conservative. If there are any adenomas >1.0 cm or any that look suspicious, mesenteric resection must be radical, with the vessels taken close to their origin.

Rectal polyps >1.0 cm should be resected either preoperatively or intra-operatively and sent for permanent or frozen section to exclude rectal cancer. If there is a degree of suspicion about the rectal polyp, this must be done preoperatively so that the surgery can be changed to IPAA if necessary.

Try to resect the terminal ileum as it flares at the entry to the cecum. This gives a wider lumen for the anastomosis.

Make sure the distal resection line is in rectum, where the tenia coli have joined together. Leaving a poorly vascularized, high pressure stump of sigmoid on top of the rectum is a set-up for an anastomotic leak or fistula.

If there is any suspicion of cancer in the colon, remove the omentum en bloc.

The ileorectal anastomosis can be done using a variety of techniques. My preference is an end-to-end handsewn anastomosis.

The mesenteric defect between the terminal ileum and the sacral promontory should be closed if possible. The small bowel can herniate through this defect. When resecting the terminal ileum, a flap of mesentery can be preserved to make this closure easy and under no tension.

Open the specimen, either in the operating room or the pathology laboratory and examine it before the abdomen is closed or the patient wakes. There may be a hard area suggesting cancer and a more extensive mesenteric resection may be needed.

11.4.1.a Postoperatively

If an IRA is done with minimally invasive technique the postoperative recovery is usually smooth, requiring a hospital stay of 2–4 days. Bowel function settles down over a few weeks to 2–4 stools a day, continence is good and urgency is minimal. Yearly proctoscopy is essential to monitor the growth of adenomas.

11.4.2 IPAA

An IPAA involves removal of the entire colon and rectum, down to the levator ani (pelvic floor). An anastomosis between an ileal pouch and the upper anus is performed. The "pouch" decreases stool frequency from >20 bowel movements per day with a straight ileo-anal anastomosis, to an average of 4–6. It works by suturing a prograde peristaltic limb to a retrograde peristaltic limb. This creates a length of bowel with twice the lumen and with no net peristaltic propulsion. The stool empties by gravity and there is no urgency. This means that the key factor determining stool frequency is pouch length, not pouch volume. There are three sets of options for the surgeon that affect the conduct of the operation: the type of pouch, the type of anastomosis, and construction of a diverting ileostomy.

11.4.2.a Type of Pouch

The most common and easiest pouch to make is the J pouch. Limbs are 15–20 cm long, but the main factor determining length is the position of the apex of the superior mesenteric artery. The stool frequency of the J pouch is similar to an S pouch, made with three 15 cm limbs. The S pouch takes longer to make than the J, as it is hand-sewn, not stapled. The Achilles heel of the S pouch is the efferent "spout," between the pouch and the anus. If this is longer than 2 cm it is prone to kink and to obstruct the stool trying to leave the pouch. The advantage of the S pouch over the J is that it will reach an extra 1–2 cm into the anal canal and that the "spout" will fit better into the anal canal than the apex of a J pouch.

11.4.2.b Type of Anastomosis

The simpler type of anastomosis is a double-stapled end of pouch to anus anastomosis. The rectum is stapled distally at the level of the pelvic floor, a purse string suture is inserted into the open end of the pouch and used to tie in the anvil of the stapler, and the anastomosis is completed by transanal insertion of the stapler cartridge, uniting the cartridge with the anvil, and firing the stapler. Residual anal transition zone is often less than 1 cm as the stapler removes 0.5–1.0 cm. Alternatively, the anal transition zone is stripped transanally (mucosectomy) and the pouch pulled down and anastomosed by hand transanally to the dentate line. The stripping and hand-sewn anastomosis takes longer and in some studies is associated with more complications and worse function than the stapled anastomosis, [9, 30], but its putative advantage is removal of all anal transitional and rectal epithelium with a more complete prevention of anal transitional neoplasia. The prime indication for mucosectomy and hand-sewn IPAA is carpeting of the anal transitional zone by adenoma.

11.4.2.c Diversion or Not

The IPAA was initially intended for use in patients with ulcerative colitis, who are often taking steroids or immunosuppressives, and have inflamed bowel. A diverting ileostomy was a routine part of an IPAA, but over time has become optional in patients at low risk for an anastomotic complication. Patients with FAP are at low risk for an anastomotic leak or fistula because they are generally healthy, are not taking immunosuppressive medications, and have normal bowel except for the adenomas. Reasons not to divert include the need for another surgery, along with the complications related to that extra operation. Furthermore, a loop ileostomy above an IPAA produces more watery and more voluminous output than an end ileostomy, making patients prone to dehydration. However, in most patients a "safety first" approach is better and the postoperative course is smoother. Function of a freshly constructed pouch is worse than that of a healed pouch, and the postoperative ileus after construction of an undiverted pouch can last several days.

The technical points of IPAA are as follows:

Preoperative colonoscopy to assess the risk of a cancer being present is important. If it is clear that there is no cancer, mesenteric resection may be reasonably conservative. If there are any adenomas >1.0 cm, or any that look suspicious, mesenteric resection must be radical, with the vessels taken close to their origin.

Rectal cancers should be carefully staged preoperatively and neo-adjuvant chemoradiation given for node positive and advanced T3 lesions. Postoperative radiation after an IPAA may lead to such bad pouch dysfunction as to require pouch removal [10].

Make a judgement about the reach of the pouch before rectal dissection begins. If the apex of the superior mesenteric artery reaches below the symphysis pubis then a stapled IPAA will be possible. However, too much tension on the anastomosis predisposes to anastomotic separation.

Techniques to extend the length of the small bowel mesentery include preserving the ileocolic arcade and dividing the superior mesenteric arcade at the tip of the pouch, making stepwise incisions in the peritoneum over the superior mesenteric artery and making an S pouch instead of a J.

If there is any suspicion of cancer in the colon, remove the omentum en bloc.

Open the specimen, either in the operating room or the pathology laboratory, and examine it before the abdomen is closed or the patient wakes. There may be a hard area suggesting cancer and a more extensive mesenteric resection may be needed.

Postoperatively

If an IPAA is done with minimally invasive technique, the postoperative recovery is usually smooth, requiring a hospital stay of 3–5 days. After ileostomy closure, bowel function settles down over a few weeks to 4–6 stools a day, continence is

good, and urgency is minimal. Yearly pouchoscopy is essential to monitor the growth of adenomas.

Indications, advantages, and disadvantages of IRA and IPAA for FAP are shown in Table 11.2.

11.5 Factors Influencing Choice of Surgery

11.5.1 Severity of Polyposis

When confronted by a patient with FAP one of the first decisions to be made is the type of operation that is most suitable for colorectal cancer prophylaxis. The main factor determining surgical choice is the severity of polyposis. This, in turn, is partially related to genotype and partially to various unknown factors such as diet and modifier genes. However, the "bottom line" is the severity of the polyposis. Patients with more than 20 rectal adenomas and more than 1,000 colonic adenomas have a greater than 50% risk of later proctectomy if their initial operation is an IRA [4]. Patients with less than six rectal polyps and less than 1,000 colonic polyps will almost always keep their rectum [4]. Patients with 6–20 rectal polyps have a relatively low chance of needing later proctecomy. If these general rules are followed, rectal cancer after IRA will be quite uncommon, although a few patients will lose their rectum to an unexpected increase in adenoma number. It is worth noting that all FAP patients need lifetime surveillance of their GI tract, even those who have had a IPAA. Adenomas develop within the ileal pouch in a majority of cases and cancers can develop in an end ileostomy, so no patient is exempt from follow-up, regardless of which surgery they have had [11, 12].

11.5.2 Patient Age and Body Habitus

Young patients are particularly susceptible to the social and developmental effects of poor bowel function after IRA or IPAA. There is a tendency of obese patients to suffer more from stool frequency and seepage after IPAA than slim patients. IPAA with stapled anastomosis is, in most hands, functionally superior to mucosectomy with hand-sewn anastomosis [13]. There is, therefore, for all these reasons, a tendency to be more conservative in young patients with factors suggesting that IPAA may be difficult or functionally suboptimal. In such patients, the "rules" may be stretched, in some cases to the point where a patient may be recommended to have a "staged" IPAA. An initial laparoscopic IRA is followed by close surveillance, rectal polypectomy where indicated, and a later proctectomy and IPAA. This same strategy can be used in patients who want to avoid the decreased fecundity associated with an IPAA, [14] or those prone to desmoid tumors.

11.5.3 Risk of Desmoids

There are several factors predisposing to desmoid tumors, such as gender, genotype, the extracolonic manifestations of Gardner's syndrome, and a family history of desmoids. These factors can be integrated into a Desmoid Risk Factor score (DRF) that allows prediction of desmoid risk [15]. The worst-behaving desmoids occur in young, nulliparous women, and these patients in particular need to be protected from early surgery if possible. The type of surgery also seems to affect the growth of desmoids. Early data suggest that while a laparoscopic IRA carries the lowest risk of desmoids, a laparoscopic pouch has the highest risk (especially in men) [8]. The data are unconfirmed but are quite compelling.

11.5.4 Timing of Surgery

The risk of desmoids is an important factor determining the timing of surgery, but the most important factor is the risk of cancer. Patients with a cancer diagnosed preoperatively should have a metastatic workup with abdominal CT scan, chest X-ray, and CEA levels. Then surgery follows as soon as convenient. Symptomatic patients generally need operation without delay, as they are at the highest risk of already having a cancer and have symptoms as well. Patients with severe polyposis also need early surgery, because of the cancer risk. In patients with mild polyposis, there is often the latitude to wait for surgery. Reasons for delay include the age and maturity of the patient, the mildness of the polyposis, financial and social considerations in the family, and risk of desmoids. While there is no evidence to show that delaying surgery reduces the risk of desmoid disease, desmoids seem to be milder in older patients and behave better in patients who have been pregnant [16].

If surgery is to be delayed, it is important that colonic surveillance continue at least yearly to avoid the possibility of a cancer developing. Surveillance colonoscopy should involve resection of any polyp >5 mm and the presence of severe dysplasia is an absolute indication for surgery. If there are no polyps >5 mm, representative polyps should be biopsied (at least 4) to test for severe dysplasia. Relative indications for surgery include a significant increase in polyp number (over 200), size (over 1 cm) and the onset of symptoms (bleeding, change in bowel habits, abdominal pain).

11.6 Outcome of Surgery

Several studies have examined the outcomes of IRA and IPAA in terms of morbidity and mortality, bowel function, quality of life, and effectiveness of cancer prophylaxis. These studies are reviewed in Tables 11.3 and 11.4. From these data, certain conclusions can be drawn.

Table 11.3 Morbidity after IRA and IPAA for FAP

		Number patients		Total complications		Leak		Obstruction	
Author	Year	IRA	IPAA	IRA	IPAA	IRA	IPAA	IRA	IPAA
Bjork [18]	2001	43	20	26%	51%				
Soravia [19]	1999	60	50	ns	ns	3%	12%	15%	24%
Nyam [20]	1997	na	187	na	24%				13%
Tonelli [21]	1997	14	24	ns	20%				
Gunther [22]		48	62	14%	27%				
Kartheuser [23]	1996	23	171	ns	27%	ns	ns	ns	15%
Krausz [24]	2005	na	174*	na	ns	na	4.8%	na	13.2%
Ambroze [25]	1992	21	94	17%	28%				
Heuschen [26]	2002	na	212			Pouch-related septic complications 9.4%			

Na Not analyzed; *Ns* Not stated; *IRA* Ileorectal Anastomosis; *IPAA* Ileal Pouch Anal Anastomosis; *FAP* Familial Adenomatous Polyposis

*146 with ulcerative colitis, 28 with FAP

Table 11.4 Bowel function after prophylactic IRA and IPAA for FAP

		n		Function/continence	
Author	Year	IRA	IPAA	IRA	IPAA
Bjork [18]	2001	43	20	Less nighttime stools, better continence and less perianal soreness	Worse
Soravia [19]		60		Better nighttime continence and less perianal irritation	Worse
Nyam [20]					4 bm/day
Tonelli [21]	1997	14	24		Night soilage in 25%
Gunther [22]	2003	62	48	Continence scores better	Worse
Kartheuser [23]		23	171	3 stools/day	4.2 stools/day
Ko [27]	2000	14	30	5.2 stools/day, no leakage, no pads, 7% perianal skin inrritation	7.5 stools/day, 43% leakage, 17% pads, 33% perianal skin inrritation
Krausz [24]	2005		28		5 stools per day
Van Duijvendijk [28]	1999	161	118	Better day and night stool frequency, less soiling, less need for antidiarrheals	
Ambrose [25]	1992	21	94	4 stools per day; no nighttime soiling	5 stools per day; night-time soiling in 4%
Salemans [29]	1992	na	72*	Patient satisfaction good in 46% of ulcerative colitis and 76% of FAP patients	

NA Not analyzed; *IRA* Ileorectal anastomosis; *IPAA* Ileal Pouch Anal Anastomosis; *FAP* Familial Adenomatous Polyposis; *51 with ulcerative colitis and 21 with FAP

1. The overall mortality in patients undergoing prophylactic large bowel surgery for FAP ranges from 0.5 to 1% [17].
2. Function after IRA is generally better than after IPAA (although some studies report equivalent function).
3. Function after stapled IPAA can be equivalent to that after IRA, although stool frequency is higher.
4. A hand-sewn IPAA predisposes to incontinence, seepage, and anal pain. It does not guarantee that there will be no anal transition zone cancer, and it creates an area of the GI tract that is difficult to survey.
5. Laparoscopic techniques offer quicker recovery, less pain, and less anxiety about the procedure.

Oncologic outcomes of prophylactic colorectal surgery in FAP have improved since the addition of the IPAA to the list of surgical options. In the "pouch era" the risk of rectal cancer after IRA is very low [4]. The risk of cancer after IPAA is even lower, [12] although as the current pouches mature the incidence of pouch polyposis and the chance of pouch cancers will rise. The type of anastomosis done after total proctocolectomy and pouch makes a difference in terms of neoplasia in the anal transition zone. This risk is 28% at 5 years after a stapled anastomosis and 14% after a hand-sewn anastomosis and mucosectomy [30]. Cancers have been reported after IPAA but are more equally divided between stapled and hand-sewn anastomoses [12]. These data reinforce the need for yearly surveillance no matter what the technique of surgery has been.

11.7 Reoperative Surgery in Patients with FAP

More than 25% of patients with FAP undergo a second abdominal surgery [6]. The most common indication for this after a prophylactic IRA is unstable rectal polyposis requiring completion proctectomy and IPAA, but currently the most common indication overall is bowel obstruction. Patients with FAP are predisposed to bowel obstruction because they seem to develop denser adhesions than patients without FAP, they are subject at least to colectomy and often to proctocolectomy with a temporary stoma, and about a quarter develop intra-abdominal desmoid disease.

11.7.1 Proctectomy and IPAA

The indication for proctectomy after IRA for FAP is an increasing instability of the rectal mucosa as evidenced by increases in the number, size or dysplasia of rectal adenomas. After an initial IRA, there is often spontaneous rectal adenoma regression, [31] presumably as a result of the constant entrance of ileal stool into a rectum used to receiving colonic contents once or twice a day. After 3–5 years adenomas tend to grow again, but can be ignored if they are <5 mm diameter or

simply snared. If rectal adenomas cannot be controlled endoscopically, or if severe dysplasia is present, proctectomy should be done.

Patients must be warned preoperatively that a restorative proctectomy may not be possible or advisable. The presence of desmoid disease is the most common cause of a failed IPAA but sometimes the small bowel mesentery is simply not long enough [6]. This is rare, but more likely to occur in obese patients. Weak sphincters or a low rectal cancer may make an IPAA inadvisable because of predictably poor function. In a desmoid-prone patient, preoperative CT scan may allow a more accurate prediction of the presence of desmoid disease, and prophylaxis with sulindac 150 mg po bid for 3 months, while unproven, may suppress desmoid tendency. Preoperative preparation includes stoma site marking.

The surgery itself involves a complete mobilization of the small bowel to the IRA. The terminal ileum just above the IRA is often considerably dilated, making for a large pouch, but the temptation to make a straight ileal-anal anastomosis with the dilated ileum must be resisted. There is no difference in function, however, between a pouch made after a secondary proctectomy and one made de novo [32, 33]. Rectal dissection can be difficult if the rectum has undergone years of polyp cautery. This creates scarring that can obliterate the planes around the bowel, can make cancers hard to detect and can reduce rectal compliance leading to urgency. For these reasons, small rectal polyps (<5 mm) are best left alone.

11.7.2 Lysis of adhesions

Bowel obstruction is the commonest reason for re-operation in patients with FAP, other than elective proctectomy. Sometimes the cause of the obstruction is a single band adhesion, often attached to the stump of the inferior mesenteric artery. Here the band is simply divided. Other patients have mesenteric desmoid disease that puckers the bowel and causes multiple sites of kinking. The kinks can be freed or bypassed, and an anti-adhesion sheet used to minimize the chance of recurrent attachment. A small minority of patients develop obliterative peritoneal adhesions creating problems in management. Adhesiolysis is painstaking, long, and technically difficult. In every case, it is important to avoid an enterotomy, as postoperative adhesions distal to the repair predispose the patient to a leak and a fistula.

11.8 Summary

Patients with familial adenomatous polyposis usually need prophylactic colectomy or proctocolectomy to prevent the development of colorectal cancer. Although many factors influence the type and timing of this surgery, the risk of cancer is the most important and influential. Patients with severe polyposis need total proctocolectomy and ileal pouch-anal anastomosis early while those with mild or

attenuated polyposis do well with a colectomy and ileorectal anastomosis. In most series, ileorectal anastomosis is safer and functionally superior to an ileal pouch-anal anastomosis. Factors that may modify the type and timing of surgery include the age and maturity (physical and emotional) of the patient, finances, family history, desire to maximize fecundity, and risk of desmoid disease. All patients need close surveillance of the residual gastrointestinal tract for life.

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Chapter 12
Duodenal Adenomas in Familial Adenomatous Polyposis

Andrew Latchford and Brian Saunders

Abstract Duodenal disease is becoming an increasingly important cause of morbidity and mortality in patients with FAP. Our current understanding of these lesions is poor and the reason for the lower malignant potential of duodenal polyps compared to colonic polyps in FAP is unexplained.

In this chapter we review our current understanding of duodenal disease in FAP, its epidemiology, aetiology and natural history. We describe the sparse evidence regarding the management of these lesions and provide our recommendations for surveillance and management.

Keywords FAP • Duodenum • Ampulla • Adenoma • Cancer • Surveillance • Treatment

12.1 Introduction

The hallmark of familial adenomatous polyposis (FAP) is the development of multiple colonic adenomas. However, in FAP there is a generalised abnormality of tissue regulation, leading to the extracolonic manifestations of this syndrome. The old names for this syndrome (familial polyposis coli and familial adenomatosis coli) neglected these manifestations, whereas the new name of FAP reflects the importance of foregut involvement.

With the exception of the oesophagus, the entire foregut is at increased risk of neoplastic and non-neoplastic polyps. The first report of duodenal disease in FAP was the post-mortem description of both gastric and duodenal polyps in a patient with polyposis [1]. However, it was almost 100 years later that the clinical importance of upper gastrointestinal tract disease was more widely recognised [2–4]. Indeed, with

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the improved outcomes in FAP, largely due to the management of the colonic disease by prophylactic surgery and the consequent reduction in incidence of colorectal cancer, duodenal adenomatosis is becoming increasingly important [5–7].

The purpose of this chapter is to describe our current understanding of duodenal disease in FAP and review the management options for affected individuals.

12.2 Epidemiology

The duodenum is the second most common site of adenoma development in FAP, with a prevalence of 30–92% [3, 8–12], and a lifetime risk of adenoma development approaching 100% [12]. The marked variation in prevalence of duodenal adenomas in the published series probably represents variation in endoscopic practice – higher prevalence rates being associated with series using side-viewing rather than forward viewing endoscopes and those protocols which included multiple biopsies [3, 10, 11].

Duodenal adenomas have been reported to appear at as early as 12 years of age; however, in general the detection of duodenal adenomas lags behind colonic polyps by 10–15 years [9, 12], although this may simply reflect the fact that upper gastrointestinal tract surveillance is not generally started until adulthood.

Duodenal cancer, along with desmoid disease, represents the most common cause of mortality in patients with FAP who have undergone prophylactic colectomy [5, 13, 14]. The risk of duodenal cancer in FAP has been estimated to be between 100 and 330 times that for the general population [4]. Estimates of lifetime risk of developing duodenal cancer of around 4% [2, 15] are largely supported by a recent, prospective multi-nation study, which set the cumulative incidence rate of duodenal cancer at 4.5% by age 57 years [12]. The cumulative incidence of duodenal cancer has been reported, however, to be as high as 10% at age 60 [16]. In these studies, the median age at which duodenal cancer developed is around 50 years.

12.3 Distribution

Adenomas may be seen in both the ampulla of Vater and also the non-ampullary duodenum. A twofold increase in prevalence of adenoma of the ampulla compared with the periampullary region has been observed by some [3], whereas in a prospective study of 102 patients, Burke et al. found an equal prevalence of ampullary and non-ampullary duodenal polyps at initial endoscopic examination [17].

Non-ampullary adenomas are most commonly seen in the second and third parts of the duodenum, with relative sparing of the duodenal bulb [11]. In particular, clustering of adenomas in the periampullary region is observed [11]. In the study by Domizio, no patients had adenomas confined to the duodenal bulb; when present here, they were always present more distally.

12.4 Aetiology

Some factors have been identified, which may be involved in the aetiology or modulate severity of duodenal disease in FAP. However, this area requires further research for clarification.

12.4.1 Bile

The distribution of polyps within the duodenum mirrors duodenal mucosa exposure to bile, suggesting a role for bile in duodenal neoplasia in FAP. Initial studies looking at the mutagenicity of bile, by the detection of point mutations in bacteria failed to reveal any difference between bile from FAP and non-FAP patients [18]. However, bile from patients with FAP has been shown to differ to that from non-FAP patients, containing a higher total biliary bile acid concentration [19]. Patients with polyposis have a greater proportion of chenodeoxycholic acid, a lower proportion of deoxycholic acid and the ratio of chenodeoxycholic acid and its metabolite lithocholic acid to cholic acid and its metabolite deoxycholic acid is higher in patients with FAP [19].

A critical step in carcinogenesis is the reaction of carcinogens with DNA to form chemical adducts. Spigelman and colleagues used 32P-postlabelling to measure DNA adducts in paired gastric and duodenal mucosa in patients with FAP and in normal controls. They observed significantly higher adduct labelling in patients with FAP and that adduct labelling was greater in the duodenum compared to stomach in patients with FAP [20]. Using similar techniques, it has been observed that rats treated with gallbladder bile from FAP patients had significantly higher adduct levels compared to those treated with bile from control patients [21]. These findings were confirmed by the same group, who incubated salmon sperm DNA with bile from FAP patients and normal controls [22]. Using reverse-phase high performance liquid chromatography, they also found similar peaks in bile from both groups, suggesting they contain similar genotoxic compounds, although the levels were higher in FAP patients, suggesting that bile from FAP patients is more genotoxic. More recently, it has been found that the dietary administration of unconjugated bile acid increases duodenal tumour multiplicity in a murine model of FAP [23]. These findings support the hypothesis that bile may be involved in foregut neoplasia in FAP.

12.4.2 Genetics

The finding of familial segregation in the occurrence and severity of periampullary neoplasia in 144 patients from 74 families with FAP [24] has raised the suggestion of a genetic influence on duodenal disease. However, it is not yet determined whether this segregation reflects the action of an unidentified modifier gene or, indeed, is not genetic and reflecting an environmental effect.

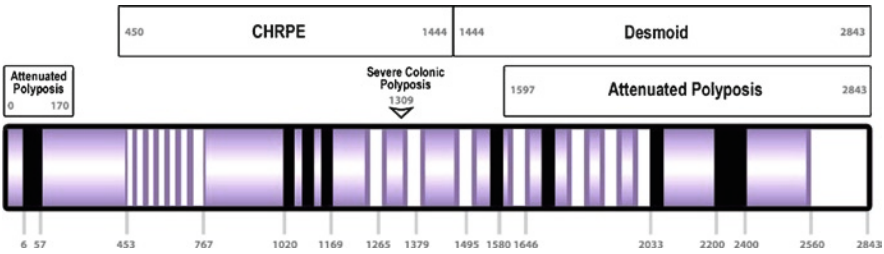

Fig. 12.1 The genotype–phenotype relationship in FAP

A family with severe duodenal disease and a germline *APC* mutation at codon 1520 has also been described [25]. Again this may reflect that this family carries a gene that modifies their risk of developing duodenal neoplasia or may be taken to reflect the effect of this particular germline *APC* mutation. Indeed a number of series have tried to address whether or not a genotype–phenotype relationship exists for duodenal disease, that is, whether specific germline *APC* mutations may reflect the clinical course of duodenal adenomatosis. A genotype–phenotype relationship has been shown to exist for a number of aspects of the syndrome of FAP (Fig. 12.1) and so it would seem reasonable to expect a similar relationship to exist with respect to duodenal disease. However, the findings in the published series are not consistent, although most indicate that mutations in exon 15 are associated with a severe duodenal phenotype [16, 26, 27]. This finding is difficult to interpret and should be regarded with caution, since exon 15 covers more than 75% of the coding region of *APC* and the majority of both germline and somatic mutations arise in exon 15 [28]. Currently the data are insufficient to make any specific management recommendations based on *APC* mutation position.

12.5 Hormones and Pregnancy

There is some evidence to suggest that hormonal factors may be involved in carcinogenesis in the upper gastrointestinal tract. Campbell-Thompson and colleagues investigated rats that had been exposed to the carcinogen N-methyl-N′-nitro-nitrosoguanidine (MNNG) [29]. They observed that exposure to MNNG caused blunting of the duodenal villi and induced hyperplasia and dysplasia in both gastric antral and duodenal mucosa. Systemic treatment with 17β-estradiol was observed to modulate pre-neoplastic changes; significantly reversing some of the MNNG-induced morphological alterations but not altering proliferation and apoptosis rates. Both rat antral and duodenal epithelium are known to express oestrogen receptors, particularly the ERβ subtype, suggesting that oestrogens may have a direct effect of on the GI tract epithelium [30, 31].

More recently, pregnancy has been shown to affect small intestine tumour multiplicity in a murine model of FAP [32]. Using two recombinant inbred lines of Min/+ mice, pregnancy was seen to be associated with increased tumour multiplicity compared to virgin controls in one line only. Interline crosses indicated that this

effect was under genetic control. There are also early data to suggest that pregnancy may be associated with an increased risk of severe duodenal disease in some patients with FAP (Latchford A., unpublished data).

These data are clearly sparse and further work in this area needs to be performed. Pregnancy is associated with a number of changes including levels of oestrogens and prostaglandins; therefore the findings presented above cannot be taken to purely reflect an effect of oestrogens. Although the data are few, if confirmed, this may have a significant clinical impact on the course of duodenal disease in females and our management recommendations.

12.6 Adenoma–Carcinoma Sequence

In the colorectum, a stepwise progression from normal mucosa to aberrant crypt foci, adenoma and through varying degrees of dysplasia to carcinoma has been described and widely accepted to be one of the mechanisms of colorectal tumorigenesis. This is termed the adenoma–carcinoma sequence. In this model, the initiating event is activation of the Wnt signalling pathway by either biallelic *APC* mutations or a somatic β-catenin mutation. Thereafter, further somatic mutations in tumour suppressor genes and oncogenes are acquired, along with alteration of the expression of important regulatory proteins.

In FAP, there is evidence that a similar pathway exists in duodenal tumorigenesis. Spigelman et al. explored the association between duodenal adenoma and carcinoma in 47 patients with FAP [33]. They found adenomatous elements either as a component of the cancer or immediately adjacent to the cancer in 38 of the 45 (84%) evaluable patients. Factors associated with malignant change included villous histology and moderate or severe dysplasia.

Patients with FAP inherit a germline mutation in the *APC* gene and a somatic mutation is required for biallelic mutation and activation of the Wnt signalling pathway. Somatic *APC* mutations were observed in 50% ampullary cancers, 67% ampullary adenomas and 46% duodenal adenomas in one study [34], but lower detection rates have been reported by others [27, 35]. Part of this variation may reflect the methodology used in the studies; for example, in the study by Gallinger et al. only the mutation cluster region of *APC* was examined, whereas in the study by Groves et al., the entire gene was examined. In addition with more modern techniques such as multiplex ligation probe amplification (MLPA), an improved detection rate may be expected.

Groves et al. reported interdependence between the germline and somatic APC mutations in duodenal adenomas, which they observed to be non-random and chosen to provide an optimal level of β catenin signalling [27]. Similar findings have been observed in both colorectal polyps and desmoid tumours in patients with FAP [36, 37].

K-Ras codon 12 mutations have been described in periampullary and duodenal adenomas and cancer in patients with FAP [35, 38] and have been demonstrated in sporadic ampullary adenomas and cancer [39]. Overexpression of p53 has been observed in duodenal tumours in FAP. Kashiwagi et al. noted overexpression of this

tumour suppressor protein in 25% of tubular, 72% tubulovillous/villous adenomas, and 100% duodenal carcinomas [40]. In normal cells, wild type p53 has a very short half-life and is usually undetectable by immunohistochemistry. It is only by stabilisation of the p53 protein (e.g., by mutation) that levels become detectable by standard assessments of expression. SMAD4 mutations have not been assessed in duodenal tumours in FAP; however, there is evidence that they be involved in upper gastrointestinal tumourigenesis in a mouse model [41] and in sporadic ampullary lesions [42].

In addition to the above mutations, other important mediators of neoplasia and its progression have been identified in duodenal tumours in FAP. Cyclo-oxygenase 2 (COX-2) is overexpressed in sporadic colorectal and gastric neoplasia. Using a semi-quantitative, five-point immunohistochemical scoring scale, Brosens et al. demonstrated an overexpression of COX-2 in both sporadic and FAP-associated colonic and duodenal adenomas, compared to normal mucosa [43]. Furthermore, the normal mucosa in patients with FAP displayed greater COX-2 expression than normal mucosa from controls [43]. In addition, transforming growth factor α (TGF-α) is expressed to a greater degree in duodenal cancers than adenomas and epidermal growth factor receptor (EGF-R) expression correlates with the degree of dysplasia in duodenal adenomas [44]. Both these molecular alterations may drive transition along the adenoma–carcinoma sequence.

Relative telomerase activity has been measured in normal mucosa and duodenal adenomas from FAP patients and in sporadic papillary cancer. Telomerase activity was seen in three of seven (42.9%) normal mucosa in FAP but no activity was seen in any of the nine specimens of normal duodenal tissue in patients without FAP. Furthermore, an increase in accordance with the progression of duodenal neoplasia was observed [45].

Although the data presented here support the hypothesis of a similar adenoma–carcinoma sequence occurring both the colon and duodenum, it is not known why the risk of malignant transformation in colonic and duodenal polyps is so different. Colonic cancer is almost inevitable without prophylactic surgical intervention, whereas the lifetime risk of duodenal cancer in FAP is around 5%, even though the risk of duodenal adenoma approaches 100% [12].

12.7 Natural History

The natural history of duodenal disease in FAP has been better defined by a number of large prospective series [12, 17, 46]. However, there are areas of confusion which remain. Most of the data quote duodenal cancer risk as a whole and do not consider ampullary and non-ampullary disease separately. Furthermore, many refer to peri-ampullary disease but fail to define what is meant by this term. Strictly, peri-ampullary tumours arise within 2 cm of the ampulla and include tumours from the pancreatic head, lower common bile duct, ampullary and peri-ampullary duodenal tumours. These need to be considered separately as they are different biological entities and exhibit different clinical outcomes depending on the origin of the tumour [47, 48].

12.7.1 Ampulla

In a prospective series from the Cleveland Clinic, Burke et al. assessed the natural history of untreated ampullary disease in FAP [17]. Of the initial 110 patients, 74 (67%) had normal morphology at initial endoscopy and over a mean of 50 months, 95 (86%) showed no morphological progression. However, of the 69 with a macroscopically normal ampulla, 37 (54%) had adenomatous foci found on biopsy. In total, 105 patients had histological assessment of the ampulla and 69 (66%) had adenoamtous changes. In this cohort of 105, no histological progression was seen in 93 (89%) over a mean follow up of 48 months. Of the 12 patients who displayed histological progression, nine developed a tubular adenoma; of the two tubular adenomas, one progressed to a tubulovillous adenoma and one to an invasive adenocarcinoma.

This would indicate stable tumours with relatively slow progression and was confirmed in a smaller series of 18 patients with 10 years of follow up, in which 16 (89%) showed no macroscopic progression and only three of twelve (25%) showed an increasing grade of dysplasia.

Kashiwagi et al. performed endoscopic and histological surveillance of the ampulla in 76 patients with FAP over a 6-year period, with a minimum of two endoscopies and a median interval between endoscopies of 44 months [49]. They found progression (defined as doubling in polyp size, increasing severity of dysplasia or increase in the villous component of the polyp architecture) in only three (4%) patients. They observed that patients with an ampullary polyp >1 cm, or the polyp being tubulovillous or villous adenoma, or containing moderate or severe dysplasia (or "major disease") were significantly more likely for the ampullary disease to progress. They suggest therefore that those with major disease may have less stable polyps, which are at highest risk of early progression. As such surveillance should be more assiduous. There are no other identified clear predictors of the progression of ampullary tumours to cancer.

12.7.2 Non-ampullary Duodenum

Prospective series, each with cohorts of more than 100 patients have also suggested a slow progression in non-ampullary duodenal disease in FAP. Groves et al. followed up 114 patients over a 10-year period, using both forward and sideviewing endoscopy, following a defined biopsy protocol [46]. They observed a slow progression of all stages of duodenal disease, with 0%, 5% 7%, 5% and 4% of stage 0, I, II, III, IV disease, respectively progressing over the period of follow up. Six patients developed cancer during the period of follow up, at a median of 6 years after entering the study and from this quoted a 36% 10-year cancer risk for those with stage IV disease, 2.4% and 2.3% for those with stage III and II disease, respectively, and 0% risk for those with stage 0 and I disease. However, the patient

who developed a cancer from stage II disease was in fact an ampullary cancer. Although two other patients were said to have had ampullary cancers, it is not clear from the text whether in fact these patients had cancer of the periampullary duodenum. If so, then this would give a 36% risk of non-ampullary duodenal cancer in those with stage IV disease and a 0% non-ampullary duodenal 10-year cancer risk for those with stage II disease. This would seem more logical, as it has been demonstrated that there is no correlation between the size, number or combination of size and number of non-ampullary duodenal polyps with the presence of ampullary adenoma [17].

In a multi-centre study, Bulow et al. prospectively assessed 368 patients, using forward viewing endoscopy and a defined biopsy protocol [12]. They too observed slow progression of disease with 7% of patients having stage IV disease at entry and of those with stages 0–III disease on entry; only 15% progressed to stage IV disease over a median follow-up of 7.6 years. In this cohort, four patients developed cancer while on surveillance: 2/27 (7%) patients with stage IV disease compared with 2/339 (0.7%) with stage 0–III disease ($p<0.01$). It is not clear whether these cancers were ampullary or non-ampullary in origin.

Burke and colleagues also saw similar results [17]. They observed no significant progression in size or number of non-ampullary duodenal polyps in 65% and 74%, respectively, and no histological progression in 89% during approximately 3.5 years follow up.

These studies should be commended but do have their drawbacks, in addition to the lack of differentiation of tissue of origin of the cancers as mentioned previously. In the study by Groves et al., two patients received endoscopic therapy during follow up and 24 patients were involved in chemoprevention trials using non-steroidal anti-inflammatory drugs [46]. Both of these may alter the natural history of polyp progression, although data to support or refute this are lacking. In the five-nation DAF study, only forward viewing endoscopy was used, which may have lead to an underestimation of the disease stage, bearing in mind the periampullary clustering of polyps that occurs in FAP, an area not well visualised with a forward viewing endoscope [12]. In this study, seven patients had undergone endoscopic therapy prior to study entry, which again may have altered the natural history of the disease by removing presumably the largest or most dysplastic lesions. It is not mentioned in this study whether patients received any chemopreventative agents. In the study by Burke et al., no endoscopic therapy was performed and therefore there should be no influence on the progression of the lesions seen [17]. However, they did not use the Spigelman staging system, making comparisons between studies difficult and furthermore do not comment on the progression of severity of dysplasia, only progression of the adenomas and their villous components, along with polyp size and number are discussed.

It is clear, therefore, that those with the most advanced disease have a greater risk of developing cancer, but that overall disease progression is slow. Although age alone is not predictive of duodenal disease severity, the cumulative incidence of stage IV disease and duodenal cancer increases with advancing age [12, 16].

12.8 Endoscopic Appearances

The appearances of the ampulla are variable. It may be normal macroscopically but with microscopic evidence of disease. The ampulla may be irregular and either white or erythematous. Finally, it may be of normal size or enlarged (Fig. 12.2).

The majority of the non-ampullary duodenal polyps are small, superficial, pale plaques. These may, however, progress and coalesce to form carpeting of the mucosa. Some polyps, however, are not plaque-like and resemble more the sessile polypoid lesions that are seen in the colon (Fig. 12.2).

Spigelman and colleagues developed a useful system for rating the severity of duodenal polyposis, using both endoscopic and histological appearances (Table 12.1) [3]. This classification describes five stages (0–IV) according to the number and size of polyps, histological architecture and severity of dysplasia. This classification has been demonstrated to correlate with the risk of duodenal malignancy [46].

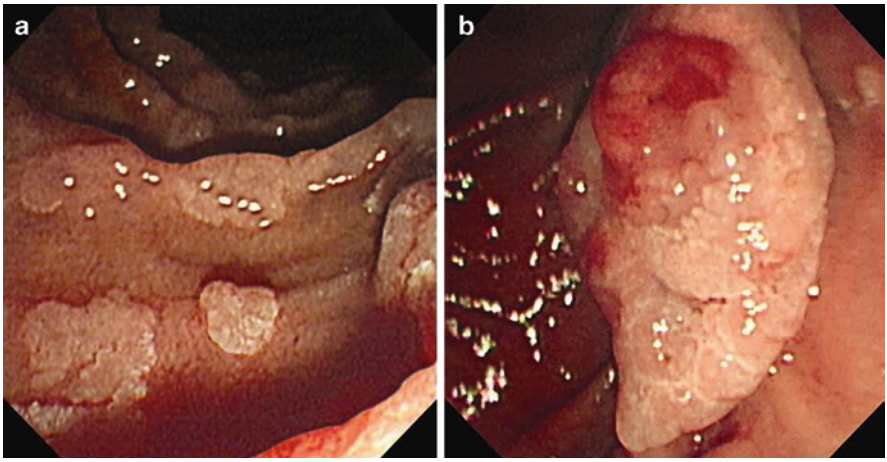

Fig. 12.2 Examples of non-ampullary (**a**) and ampullary (**b**) duodenal lesions

Table 12.1 The Spigelman classification of duodenal polyposis

	Points		
Criterion	1	2	3
Number	1–4	5–20	>20
Size	1–4	5–10	>10
Architecture	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage 0: 0 points, Stage I: 1–4 points, Stage II: 5–6 points, Stage III: 7–8 points, Stage IV: 9–12 points

However, this classification does have some drawbacks, largely relating to polyps in the ampulla. Sporadic ampullary and duodenal adenomas behave differently and have different prognoses if cancers arise [47, 48]. Furthermore, in FAP, there is no correlation between non-ampullary duodenal disease and the presence of ampullary adenoma [17], supported by preliminary evidence suggesting that the median Spigelman stage from which cancer arises, is less for ampullary than non-ampullary tumours [50]. Therefore, the Spigelman classification cannot really be used to stage ampullary disease and will not reflect the risk of malignant progression.

12.9 Management

12.9.1 Endoscopic Surveillance

Due to the improved understanding of duodenal disease and its natural history, surveillance of the upper gastrointestinal tract is recommended by most authorities, although the frequency and mode of surveillance recommended may differ. However, the need to adjust the frequency of surveillance according to the severity of the disease is recommended uniformly and the Spigelman classification system provides a framework upon which this can be done.

There are no uniform recommendations regarding surveillance for ampullary disease, in terms of frequency. Ampullary disease risk is not reflected by the non-ampullary polyps [17]; therefore, the Spigelman classification does not seem a logical choice to determine ampullary surveillance intervals. Therefore, when deciding on surveillance intervals, both ampullary and non-ampullary disease should be taken into account.

In addition to endoscopy frequency, surveillance biopsy protocols differ. We would recommend that any polyp greater than 1 cm, or those with worrying macroscopic appearances, should be biopsied. Even if all lesions are small and benign looking, representative biopsies of the largest lesions should be taken, as it has been reported that the polyp size and/or number do not reflect histology in non-ampullary disease [17] and albeit anecdotal, we have detected severe dysplasia in non-ampullary polyps as small as 6–7 mm.

One problem of surveillance biopsies is that they are representative and therefore can lead to sampling error. This has been highlighted in a worldwide survey among polyposis registries of the surgical management of severe duodenal adenomatosis in FAP [51]. Of the 56 patients operated on for stage III or IV disease, a higher degree of dysplasia was detected in the surgical specimens of 11. Furthermore, ten patients with a preoperative diagnosis of severe duodenal adenomatosis were found to have invasive cancer in the resected specimen, a finding borne out in other series [52]. This supports the earlier findings of Nugent and colleagues [53], who found that an increase in the number of biopsies correlated with an increase in the degree of dysplasia and villous histology.

12.9.2 Medical

The majority of the patients with FAP will develop duodenal adenomas, but only a small proportion will progress to advanced duodenal disease or duodenal cancer. As such, universal prophylactic major resectional surgery, as is performed for colonic disease, is not indicated. In addition, there are a proportion of patients for whom surgery may be indicated but rendered impossible, for example, due to presence of significant intra-abdominal desmoid disease. As such, interest has arisen in exploring pharmacological and endoscopic therapies for duodenal adenomatosis.

There are good data that non-steroidal anti-inflammatory drugs (NSAIDs) can cause regression of colorectal adenomas in FAP patients [54–56]. Consequently, much of the work looking for pharmacological therapies for duodenal adenomas has focused on NSAIDs. Sulindac has been studied in the setting of a randomised, placebo-controlled trial [54], a randomised controlled trial [57], a non-randomised clinical trial [58] and finally a controlled, non-randomised, dose-finding study utilising sulindac suppositories [59]. All these studies contain small numbers of patients and all failed to demonstrate sulindac-induced regression in duodenal disease. More recently, studies have assessed the efficacy of the selective COX-2 inhibitors. Preliminary results from a study of 12 FAP patients, with the control group receiving ursodeoxycholic acid, demonstrated a beneficial effect in two patients out of the six treated with rofecoxib [60]. More robust data comes from a large, adequately powered, randomised trial by Phillips and colleagues [61]. After 6 months of therapy with celecoxib 800 mg/day compared with placebo, a 14% decrease in polyp burden was seen with celecoxib, although this was not statistically significant. However, paired assessment of endoscopic videotapes revealed a significant difference ($p = 0.033$) between the two arms and in subgroup analysis of those with stage III/IV disease at the outset, a 31% reduction in polyp area was observed. Long-term data on the benefits of celecoxib are not available and it is not clear whether it alters the natural course of the disease or modifies duodenal cancer risk. In clinical practice, a wide variation in response to celecoxib is observed among patients. This may be due to COX-2 promoter polymorphisms that alter duodenal mucosa COX-2 expression [43] or due to candidate polymorphisms (e.g., CYP2CP) that may modulate response to the drug [62] as has been demonstrated with COX 1 [63]. This genetic variability may be exploited to identify those who may specifically benefit from therapy or those who may be at a higher risk of potential cardiovascular complications.

As described earlier, bile has been implicated in duodenal tumourigenesis in FAP, due to increased DNA adduct formation. 32P-postlabelling in vitro experiments have shown that the excess adduct labelling is pH sensitive [64] and on the basis of this gastric acid-lowering therapy with ranitidine has been studied in 26 patients with FAP [65]. Despite the promising in vitro findings, this study failed to show a benefit of ranitidine in reducing duodenal polyp burden or in relative adduct labelling. Despite the potential importance of bile acids in duodenal adenomatosis in FAP, there are currently no placebo-controlled data assessing the effect of ursodeoxycholic acid.

Work in a murine model of FAP has suggested that the combination of an NSAID with difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, may be more effective than NSAID monotherapy, in terms of adenoma prevention [66]. This is the subject of an ongoing multi-centre randomised trial. Furthermore, there is early, non-randomised data in five patients, suggesting that combination treatment with curcumin and quercetin reduces the number and size of ileal and rectal adenomas in patients with FAP [67]. Randomised controlled trials are needed to validate these findings.

12.9.3 Surgical

To date, there are no published, randomised studies to give an evidence base upon which to base surgical selection for duodenal disease in FAP. There have, however, been a number of published single centre series as well as the results of a worldwide survey.

Initial surgical management of non-ampullary duodenal adenomatosis included local resection, by duodenotomy and polypectomy under direct vision. This approach has been abandoned, since recurrence occurs uniformly after 12–53 months and the risk of postoperative complications exists, including duodenal leaks in 2/7 (29%) in one series [51, 68–70].

Local resection of the ampulla has been reported with more varied results. In a worldwide survey of polyposis registries, eight patients were reported to have undergone ampullectomy, with one case of minor morbidity but recurrence in 6/8 (75%) after a mean follow up of 11 months [51]. Duodenotomy and ampullectomy was also associated with recurrence in the four FAP patients reported by Soravia, although no major morbidity was noted [71]. In contrast, Ouaissi and colleagues have reported their outcomes from formal surgical ampullectomy: resection of the biliopancreatic junction and of some pancreatic head tissue and reinsertion of the common bile duct and pancreatic duct into the duodenal wall [72]. One patient had a duodenal fistula at day 8, which was treated successfully by conservative measures but no other major or minor peri-operative morbidity was observed. One patient developed a pancreatic anastomotic stenosis, with consequent pancreatitis, 4 years after surgical ampullectomy. No recurrent disease was noted during a mean follow-up of 58 months.

More radical resection is indicated for patients with stage IV disease, failed local/endoscopic therapy or in those in whom carcinoma has arisen. There is debate as to whether those with stage III disease should also be considered for radical resection, bearing in mind their lower long-term risk of developing duodenal cancer and the potential major morbidity or mortality associated with such procedures. However, it is clear that surgical intervention should occur before cancer arises, as the prognosis in this group is poor, with 51–80% patients dying at an average of 19–29 months postoperatively [51, 52]. The type of surgery, be it a traditional or pylorus preserving pancreatoduodenectomy, or a pancreas preserving duodenectomy, will largely depend on local expertise. A summary of the published series is shown in Table 12.2.

Table 12.2 Summary of major resectional surgical outcomes for duodenal disease in FAP

Author	Surgery	No. of patients	Post-op complications
Marpugo [82]	PD	4	1 major, 4 minor
Alarcon [83]	PSD	3	Not stated
Penna [84]	PD	12	2 major
De Vos tot Nederveen Cappell [51]	PD	23	12 major, 5 minor. 1 post-op death
	PSD	6	3 major, 1 minor
	PPPD	12	4 major, 1 minor
Ruo [85]	PPPD	7	1 major
Chung [86]	PSD	4	1 major, 1 minor
Kallady [87]	PSD	3	1 major, 1 minor
Balladur [88]	PPPD	2	Not stated
Gallagher [52]	PPPD	16	11 major
Mackey [73]	PSD	21	12 major, 2 minor

The two largest single centre series addressing surgical outcomes in this group show quite variable results. In the St Mark's series of 16 patients undergoing pylorus-preserving pancreatoduodenectomy, eight patients suffered 11 major postoperative complications, and of the 16 patients, only nine were alive at a mean of 38 months after surgery [52]. The series from the Cleveland Clinic reported a cohort of 21 patients who underwent pancreas-sparing duodenectomy [73]. They observed no mortality and 14 complications (major and minor) in eight patients (38%). In this series, however, only one patient who underwent surgery had duodenal cancer, which may explain in part the differences in survival between the two series.

Postoperative endoscopic surveillance is still required in those that have undergone surgery, as polyps or cancer may arise in a duodenal remnant [74, 75] or in the jejunal limbs [73].

12.9.4 Endoscopic

Endoscopy provides the opportunity for multi-modal therapy for both ampullary and non-ampullary duodenal lesions in FAP. It is minimally invasive compared to surgical therapy and repeated procedures can be performed relatively easily. Endoscopy therapy (Fig. 12.3) may include simple snare resection, endoscopic mucosal resection (EMR), thermal ablation or argon beam plasma coagulation and photodynamic therapy (PDT). The eradication of duodenal polyposis is not attainable using these techniques and undertaking therapy with this aim can only lead to disappointment. By targeting those lesions that are deemed to be high risk, on size or histological criteria, control of duodenal disease may be achieved and major surgery avoided or at least delayed, although there are no long-term data to verify that such intervention alters the natural history of the disease, specifically cancer risk.

There are few data published on series containing large number of patients. The largest series by Norton et al. contained 59 patients with FAP and 32 sporadic

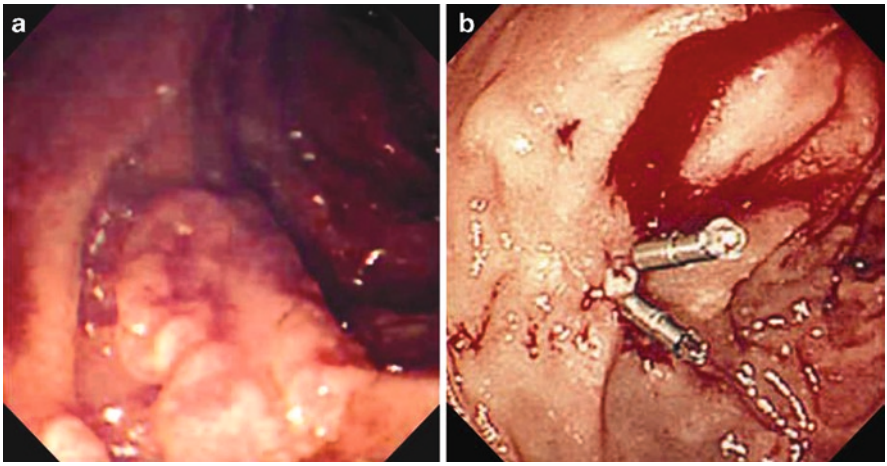

Fig. 12.3 A duodenal polyp removed by endoscopic mucosal resection and endoclip application to treat bleeding after resection

lesions [76]. Periampullary disease was treated using thermal and Nd-YAG laser ablation and snare ampullectomy. A return to normal histology was achieved in 34% of those with FAP during a median follow-up of 24 months. In this series, three patients suffered major complications and 12 patients suffered minor complications. Others have observed less satisfactory results of endoscopic resection of the ampulla. In a series of 103 patients, of which 31 had FAP, those with FAP were significantly less likely to have successful procedure compared to those with sporadic tumours and more likely to require surgical intervention for recurrent disease [77].

In a recent study at St. Mark's, 47 patients have undergone endoscopic therapy for non-ampullary disease, with endoscopic follow-up data on 29, with a median follow up of 24 months (range 3–111) [78]. Eighty-five therapeutic procedures have been undertaken and 90 lesions treated. When assessed by Spigelman staging, 12 patients have been downstaged, three progressed and 14 unchanged. No cases of cancer have arisen and two patients referred for surgical assessment due to the finding of severe dysplasia and diffuse, advanced disease. In this series, there were no cases of procedure-related mortality nor perforation. There have been ten cases of haemorrhage, of which four required transfusion and two required endoscopic therapy.

Although the data suggest that these endoscopic therapies are safe, longer-term data are required before we can adequately counsel patients regarding the risks and benefits of endoscopic therapy as opposed to early surgical intervention.

Photodynamic therapy, involves the oral administration of a photosensitising drug, followed by the endoscopic application of light. The few data that are available in sporadic and FAP-associated lesions are disappointing; with minimal reduction in tumour bulk and the long period of sensitisation and the need for repeated endoscopies making PDT poorly tolerated [79, 80]. This method cannot be generally recommended.

12.9.5 Summary

This summary contains our recommendations based on the current understanding of the disease and the relatively sparse data available.

We would advocate starting surveillance at 25 years of age, with a sideviewing endoscope and performed by endoscopists experienced in managing patients with FAP. Biopsies should be taken of any lesions >1 cm in size or if worrying appearances (such as ulceration and bleeding), if none are larger than 1 cm then representative biopsies of the larger lesions. If the ampulla appears normal we would not routinely perform biopsies, due to the risk of iatrogenic pancreatitis [81], but consider them mandatory if macroscopically abnormal.

We perform surveillance every 5 years for those with stage 0 or I disease, 3 yearly, 1 yearly and 6 monthly for those with stage II, III and IV disease, respectively. If the ampulla were macroscopically abnormal, we would consider annual surveillance, even if there were minimal non-ampullary disease.

Our indications for endoscopic therapy for non-ampullary lesions is any lesion greater than 1 cm in size or if severe dysplasia is found in relatively isolated disease. If the ampulla were greater than 1 cm, we would recommend staging by endoscopic ultrasound and CT with a view to performing ampullectomy (endoscopic or formal surgical excision) in the absence of advanced non-ampullary disease. Patients with stage III disease are counselled regarding the initiation of celecoxib 800 mg daily, either as monotherapy or in conjunction with endoscopic therapy.

Surgery is considered in patients with stage IV disease or in those who have failed the above approaches or in whom endoscopic therapy is not possible and requires a multidisciplinary approach involving endoscopists, surgeons, pathologists and radiologists.

It is clear that the primary aim is to prevent cancer and the difficult decisions facing the clinician are weighing up risks of developing cancer versus the risks and benefits of the above therapeutic strategies, in the absence of high quality data on which to make these decisions.

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Chapter 13
FAP-Associated Desmoid Tumours

Gabriela Moeslein

Abstract Desmoid tumours are rare and may occur spontaneously or in association with FAP (familial adenomatous polyposis). In both settings trauma as a trigger for growth initiation has been postulated. They differ in their predominant localisation, whereas differences in biological behaviour or response to therapeutic approaches have yet to be delineated. In all cases the tumour is frequently not recognized and treatment options are not being weighed thoroughly. Frequent debulking procedures lead to more aggressive growth and high morbidity and mortality. For the majority of desmoid tumours a less aggressive therapeutic approach may be more benefical in the long-term results, especially for desmoids occurring in the mesentery of FAP patients, that have already been subjected to colectomy.

Keywords Familial adenomatous polyposis (FAP) • Extracolonic manifestation • Desmoid tumour • Aggressive fibromatosis

13.1 Introduction

Desmoids are rare tumours of fibroblastic origin which may rarely occur sporadically or more frequently in the context of familial adenomatous polyposis (FAP), occurring in 15–30% of patients. Synonymously referred to as aggressive fibromatosis, desmoids are a major source of morbidity and mortality in FAP patients, specifically after prophylactic colorectal surgery. Predisposing factors are a distal germline mutation in the APC gene, a family history of desmoids and external factors such as trauma and most probably oestrogens. Since most FAP-asociated desmoids are apparently triggered by inevitable surgical trauma, apart from aiming at a reasonable delay in the timing of prophylactic surgery specifically in desmoid

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prone FAP families, there may be a benefit in chemoprophylaxis by perioperative preventive administration of antiestrogens and/or NSAIDS.

Desmoid tumours are continuing to be poorly understood. Consequently, several medical approaches have been combined with or without surgical resection or radiation therapy with controversial results. In addition, desmoids tend to regress spontaneously, if not treated surgically. Therefore, all claims of successful intervention must be critically evaluated. Desmoids are the greatest remaining challenge in the management of FAP and further research into their aetiology, classification and treatment needs to be combined with multi-centre clinical trials to improve management of the disease.

13.2 Aetiology

"Desmoid" is derived from the Greek word "desmos," meaning band-like [1]. These tumours are defined as benign fibrous tissue tumours arising in the musculoaponeurotic structures throughout the body. They do not metastasize, but are locally invasive [2]. Histologically, mature fibroblasts of uniform size and shape are observed, with mitosis being unusual.

Although rare in the general population with an annual incidence of only 2–4 cases per one million population [3], desmoid tumours are a common extracolonic manifestation of familial adenomatous polyposis (FAP). Approximately 2% of all desmoid tumours are associated with FAP, and patients with FAP have a 1,000-fold increased risk of developing desmoid tumours, compared with the general population [4]. The association between desmoid tumours and FAP first was made in 1923 by Nichols [5]. In 1951, Gardner [6] reported the familial occurrence of intestinal polyposis, osteomas, fibromas, and epidermal or sebaceous cysts. In 1958, Smith [7] proposed the name of Gardner syndrome for this clinical entity. The current view is that Gardner syndrome is a random variant of the phenotypic expression of FAP [8]. As screening for FAP becomes more efficient, the relative importance of extracolonic manifestations of FAP has increased [9].

Although the aetiology of desmoids remains unknown, at least three factors are associated. Most importantly, there is a genetic predisposition in the FAP population. Taking the frequency of desmoids under this condition into account, a mutation in the APC gene and therefore β-catenin levels within the cell are relevant for desmoid pathogenesis. The APC gene regulates the level of β-catenin, a mediator in the wingless (Wnt) signalling pathway, and is involved in cell adhesions [10, 11]. Elevated β-catenin levels are seen in desmoids, and it appears that β-catenin binds to and activates transcription cell factor-3 (TCF-3) [12]. Secondly, endocrine factors play an apparent aetiological role: the female-to-male prevalence ratio of 3:1 and the increased frequency of desmoid tumours during or after pregnancy led to this assumption. In the meantime, the well-established success of antiestrogen therapy in the treatment of desmoids underlines this association even if the effect appears to be independent of the oestrogen receptor status in the tumour. Immunohistochemical staining has demonstrated that desmoids are negative for the α-oestrogen receptor, human epidermal growth factor-2 (HER2), and

the progesterone receptor; however, these tests yielded positive results for the androgen receptor and, in some cases, for the β-oestrogen receptor [13].

The third factor associated with desmoid tumours is physical trauma – the main underlying cause for sporadic, non-FAP associated aggressive fibromatosis. In the setting of FAP, prophylactic surgery is the predominant trauma that triggers desmoid growth and the explanation for the infrequent occurrence of desmoids prior to colorectal surgery.

13.3 Categorization and Staging

Some major issues when considering treatment for this tumour are pointed out in Table 13.1.

Keeping these issues in mind, obviously, the major reasons for the most heterogeneous reports encountered in literature referring to the desmoid problem are mentioned above, in addition to the fact that clearly some desmoids regress spontaneously. In the abundant single case reports encountered in literature several of the above-mentioned treatment options have sequentially or simultaneously been administered to patients. Reports of successful therapy in this situation are impossible to be attributed to and correlated with one or the other regimen. Location of desmoids, size at diagnosis, time elapsed since colectomy of FAP patients, previous surgery – specifically desmoid resection – margins and other important factors are often missing in reports from less desmoid-experienced institutions.

13.3.1 DES Classification (Diameter–Expansion–Site for DESmoids)

The first published attempt to our knowledge suggesting a classification of desmoid tumours was made by our group in 2000 [14] and during the LCPG/ICG meeting in Venice. This proposed classification has been successfully evaluated in our series of 118 desmoid patients (unpublished data) Table 13.2.

Table 13.1 Major issues when considering treatment for desmoid tumours

FAP-associated versus sporadic desmoid
Location in the mesentery, abdominal wall or extra-abdominal desmoid
Invasive therapy (R0 wide margin, R0 ,R1 ,R2)
Treated medically with or without chemotherapy
If chemotherapy: aggressive or less aggressive protocol
Treated with or without radiation
Time elapsed between diagnosis and treatment
Speed of growth at initial diagnosis
Site of mutation if FAP-associated
Combined surgery and other therapies

Table 13.2 Suggested "DES classification" for all desmoid tumours [14]

	D (=diameter) size in cm/inches	*E* (=expansion) rate of doubling size in months	S (=site) location
0	Minimal lesion (desmoplastic reaction)	Unknown at diagnosis	Unknown
1	<5 cm	>24 months	E
2	5–10 cm	12–24 months	AW
3	10–20 cm	6–12 months	M1
4	>20 cm	1–6 months	M2

E extraabdominal; *AW* abdominal wall; *M1* mesentery without obstruction; *M2* mesentery with obstruction

The advantage of this classification score lies in the fact that it may be used for all desmoids and gives a good estimate in regards to the malignant biological potential. Classification in this form will be a valid aid to clinicians when deciding on how aggressive the therapeutic concept selected should be.

A different classification has more recently been proposed by Church et al. [15].

13.3.2 Desmoid Tumour Staging System [15]

Stage	
I	Asymptomatic, <10 cm maximum diameter, and not growing
II	Mildly symptomatic, <10 cm maximum diameter, and not growing
III	Moderately symptomatic or bowel/ureteric obstruction, or 10–20 cm, or slowly growing
IV	Severely symptomatic, or >20 cm, or rapidly growing

Mildly symptomatic = sensation of mass, pain, but no restrictions; moderately symptomatic = sensation of mass, pain, restrictive but not hospitalised; severely symptomatic = sensation of mass, pain, restrictive and hospitalised.

An evaluation of this proposed classification in a series of patients with mesenteric desmoids has now been reported [15]. The authors conclude that desmoids staging with this scoring system identifies tumours by prognosis and its use for designing prospective studies is reasonable. It may, however be critically remarked that this scoring system is valid only for mesenteric FAP-associated desmoids. Also, only stage IV according to this classification may be rapidly growing. However, ideally in an FAP follow-up setting small but fast-growing desmoids specifically need to be identified in order to select a more aggressive therapy before the desmoids reaches the size noted in this category of 20 cm in diameter.

13.3.3 MRI Appearance as a Predictor of Growth Potential

When a newly identified tumour is submitted to diagnostic procedures, the pattern usually is through imaging: X-ray examination (XR), ultrasonography (US),

computed tomography (CT) and magnetic resonance imaging (MRI). These examinations may be helpful to the clinician for tumour management, preoperative planning and for follow-up. On X-rays, lesions may be undetectable or show a specific soft-tissue mass, whereas CT depicts a hypo-isodense mass with contrast-enhancement. MRI examination is often the method of choice; however, recent reports do not confirm previously suggested prediction of biological behaviour based on signal intensity on T2-weighted images. Also, although there are no obvious histological or signal intensity differences on MRI, desmoid recurrences are often more aggressive than primary lesions with more spread extra-compartmentally and bone involvement [16].

Although MRI is the best imaging modality for the time being, it is now considered more useful for lesion characterization (shape, contours, margins, signal intensity) than for predicting tumour behaviour. In literature [16], the natural history of aggressive fibromatosis has two linear phases: on T2-weighted sequences, an increase in size with high signal intensity followed by a decrease in size associated with a decrease in signal intensity. Thus, a high signal was interpreted as an index indicative of an increase in size, while areas of low signal indicated a regression in lesion size over time.

Recently, Castellazi et al. [17] found no direct correlation between size and signal characteristics. Despite different signal characteristics at the beginning, ranging from white to black was observed; some desmoids increased in size despite the stable signal.

Moreover, some lesions remained stable in size with a low initial signal increasing over time, or with very high initial signal decreasing over time. Some lesions increased in size with a high initial signal followed by a decrease. The authors conclude that:

1. Fibromatoses are soft-tissue tumours with an extremely high variability in signal and size.
2. Their behaviour cannot be predicted based on their initial MRI signal.

13.4 Treatment

13.4.1 Radiotherapy

Radiotherapy has been a first hour therapy for desmoid tumours. Treatment for desmoid tumours has in this regard been based on the rationale for low-grade soft tissue sarcomas. This is due to the categorization of desmoids into this group [18, 19]. Despite their local aggressiveness, however, extraabdominal fibromatoses are usually not life-threatening. Therefore, arguably, treatment should be different from that for sarcomas and other cancers. For example, quality of margins, which is of utmost importance in sarcoma, shows contradictory results in desmoids [20–23].

However, due to the predominant localisation of FAP-associated desmoids in the mesentery of the abdomen, rare thought is spent on employing this modality. In our own hands, for very selected patients with fast-growing desmoids – also in the setting of abdominal desmoids – radiotherapy has been successful with low morbidity and low recurrence. However, we have always combined radiotherapy with a previous attempt of high-dose antiestrogen treatment in combination with sulindac and have continued medication throughout radiation and long-term thereafter. Radiotherapy still is a frequent recommendation for patients in combination with surgery in the treatment of desmoids occurring on the extremities.

However, functional impairment and recurrence are frequent without additional medical treatment.

13.4.2 Surgery

Surgery remains a useful modality of therapy for a small proportion of desmoids, although with defined indications. Surgery of abdominal wall desmoids is generally safe: in a series at St Mark's, 51 abdominal wall desmoids were excised with no mortality or significant morbidity, but 41% recurred. Surgery for mesenteric desmoids is more contentious; in the same series, 36% of patients operated on for mesenteric tumours died in the perioperative period from haemorrhagic complications and nearly half of the survivors needed extensive enough enterectomy (due to small bowel involvement) to require long-term parenteral nutrition. In addition, the tumour recurred in 71% of these patients [24]. Other series report similar rates of recurrence and risk for surgery on intra-abdominal tumours.

Surgery may be an option for small fast-growing abdominal wall tumours, if and when they may be removed with a wide margin without causing a too large defect/morbidity. However, in our experience these desmoids usually will respond to medical treatment, which implies that they have a much lower recurrence rate. In our experience, heroic attemps to remove large mesenteric desmoids result in an unacceptable risk of mortality and morbidity.

13.4.3 Systemic Therapy

Taking into account the high level of morbidity and mortalitiy with the first two described treatment options, pharmacological agents should be the initial agents of choice when deciding on treatment for desmoids in FAP. In our own series, we have been able to demonstrate that desmoids categorised in the DES treatment group 0 and 1 do not require any treatment and may be merely observed, since the variable natural history of some desmoids show spontaneous regression. The interpretation

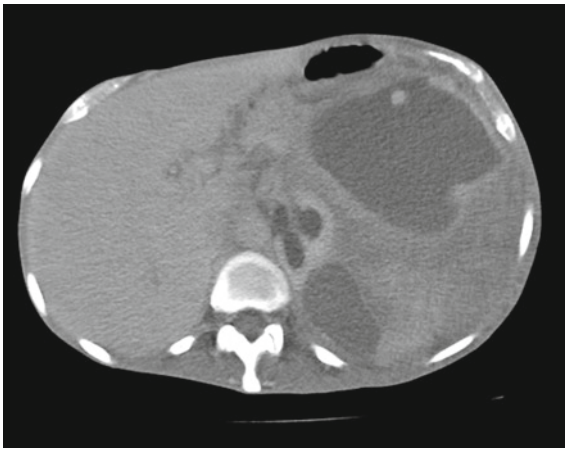

Fig. 13.1 Desmoid after therapy with high-dose tamoxifen and sulindac Chap. 13

of the efficacy of pharmacological treatments is extremely difficult due to the heterogeneity of previously treated desmoids and the lack of prospective randomised trials. We consider non-steroidal anti-inflammatory drugs (NSAIDs) and high-dose anti-oestrogens to be the first-line therapy with a high success rate. Frequently, this medication even in very large mesenteric desmoids may lead to liquefaction of the tumour due to central necrosis. In this situation, we prefer to place a drain percutaneously, which leads to immediate mechanical relief (Fig. 13.1).

However, it must be stressed that the time interval required to induce cessation of growth may require many months or even more than a year. Cytotoxic chemotherapy or radiotherapy may be required additively for DES stage 3 or 4 tumours, although continuing non-cytotoxic therapy.

Other therapies reported for the treatment of desmoid tumours with mixed results include chemotherapy with doxorubicin-based combinations [15], antiestrogen therapy with tamoxifen, [16, 17] testolactone (which inhibits steroid aromatase activitiy) and its consequent reduction in oestrogen synthesis, [18] nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin and sulindac, [19, 20] radiotherapy, [20, 21] and colchicines. [22] It is evident from all these medical alternatives that to date there is no established or evidence-based approach for the treatment of this neoplasm.

13.4.3.1 Non-Cytotoxic Chemotherapy

Apart from case reports using a variety of agents without a serious rationale, the mainstays of non-cytotoxic chemotherapy in desmoid disease are NSAIDs (most consistently sulindac) and low or high dose anti-oestrogens (tamoxifen or toremifene), used infrequently alone and commonly combined.

NSAIDs

Initially, non-steroidal anti-inflammatory drugs have been shown to reduce the incidence of both upper [25] and lower [26] GI tract polyps in FAP. Based on this experience, the ex iuvantibus assumption was that these agents could prove to be beneficial for tumours arising from similar molecular mechanisms.

The drug that has been used most in FAP-associated desmoids is sulindac. This agent has been broadly administered in FAP-associated polyp studies of the colorectum. As with all therapies, evidence for efficacy of this drug for desmoids is based on small non-randomised case series. The bias remains that the natural behaviour of these desmoids without treatment is unknown. For example, Tsukada et al. noted a 57% overall response rate to sulindac therapy in 14 FAP patients with desmoids, although the effect was typically delayed [27]. In our own series published in 2004, we confirmed the delayed response in correlation to precious therapy: surgically treated desmoid tumours were less responsive to therapy with a typically delayed time-interval for response [28]. An update on our series recently confirmed this observation in a series of now 118 prospectively treated desmoid patients in a mixed group of sporadic and FAP-associated desmoid tumours (submitted).

Other promising agents are selective COX-2 inhibitors. A study by Poon [29] demonstrated that desmoids from APC1638N mice had elevated levels of cyclo-oxygenase 2 (an enzyme inhibited by NSAIDs). When these mice were crossed on to a Cox-2 deficient background, the average tumour size was smaller, even though the number of tumours remained equal. Additionally, in cell cultures derived from human sporadic desmoids, Cox-2 blockade resulted in reduced cell proliferation.

Given the fact that the effect of cyclo-oxygenase in desmoids seems to be mediated via a Cox-2 specific mechanism, it is possible that Cox-2 specific NSAIDs such as celecoxib will have the same efficacy as drugs like sulindac with fewer of the gastrointestinal side effects. These drugs may therefore be an option in patients who have suffered side effects from the traditional drugs.

Anti-oestrogens

There is good evidence that desmoid tumours are sensitive to the effect of oestrogens, based on observations of increased incidence and growth rates in women (with highest growth rates in pregnancy) and the effects of anti-oestrogens on cell proliferation in cultures of desmoid tumour cells. Anti-oestrogen therapy has therefore been an early therapeutic agent in the treatment of desmoid tumours. The dosage administered, however, has been widely spread. Drugs used include the oestrogen receptor antagonists tamoxifen, raloxifene or toremifene. Tamoxifen has traditionally been given at doses equivalent to that used for breast cancer (i.e., 20 mg per day), but in the past years has increasingly been replaced by high-dose therapy based on good results in prospective non-randomised studies [28]. In this setting, it is impossible to ascertain what the effect would have been without treatment, but the majority of tumours either partially or completely regress under this therapy.

Recently, an Italian study investigated the effects of raloxifene on 13 patients with FAP-associated desmoids [30]. Raloxifene is an oestrogen receptor modulator, with either oestrogenic or anti-oestrogenic activity, depending on the tissue. In this small series, five patients showed complete remission of their tumours and eight patients partial remission, with no significant side effects. On the basis of these studies, further trials in studies with larger patient numbers are warranted. A review analysing the evidence for the efficacy of anti-oestrogens based on a number of non-placebo-controlled trials, many of which have reported disease stabilisation or regression, has been published by Janinis et al. [31].

Other Non-cytotoxic Agents

Other drugs have been singularily used in desmoid's disease in the past including cyclic AMP inhibitors and interferon α. The use is infrequent and results heterogeneous. Recent small trials have investigated the efficacy of the oral anti-fibrinolytic agent pirfenidone [32], a promising antifibroblastic agent with multiple impacts on inflammation via TNF beta and FGF. The tyrosine kinase inhibitor imantinib mesylate [33] activity seems mediated by receptor PDGFb and not c-Kit [34, 35]. Only few FAP-asociated desmoids have so far been included in treatment studies with imantinib. Recent reports suggest that I selected patients, this therapy may be beneficial. Due to small patient numbers, interpretation of results is difficult, and further evaluation is necessary to decide whether formal clinical trials of these compounds would be worthwhile.

Cytotoxic Chemotherapy

Once again, there are no randomised prospective trials of cytotoxic chemotherapy for desmoids in FAP, since regimens have been based largely on single arm retrospective studies.

Single agents have been used rarely, although there are case reports of tumour response to doxorubicin alone [36, 37]. More commonly, doxorubicin has been combined with another agent such as dacarbazine or cyclophosphamide and vincristine [31]. The overall response rate to doxorubicin-based chemotherapy is 50%, but at the expense of severe side effects (especially nausea and vomiting) and acute and delayed toxicity, with cardiotoxicity being a particular concern. Alternatively, the so-called low-dose chemotherapy using a vinca alkaloid in combination with methotrexate has been used with apparently good efficacy in non-FAP related desmoids [29, 38, 39]. Toxicity is less severe than with doxorubicin-based therapies but some patients experience myelotoxicity. For this reason, cytotoxic chemotherapy is probably best reserved as a second-line therapy for highly aggressive tumours (DES 3 or 4) with rapid progression or causing increasing obstructive symptoms. In our limited experience with this necessity, continued administration of our preferred medical therapy (combination of anti-oestrogen and sulindac) is beneficial to avoid recurrence that is not infrequent for tumours that have regressed after cytotoxic therapy.

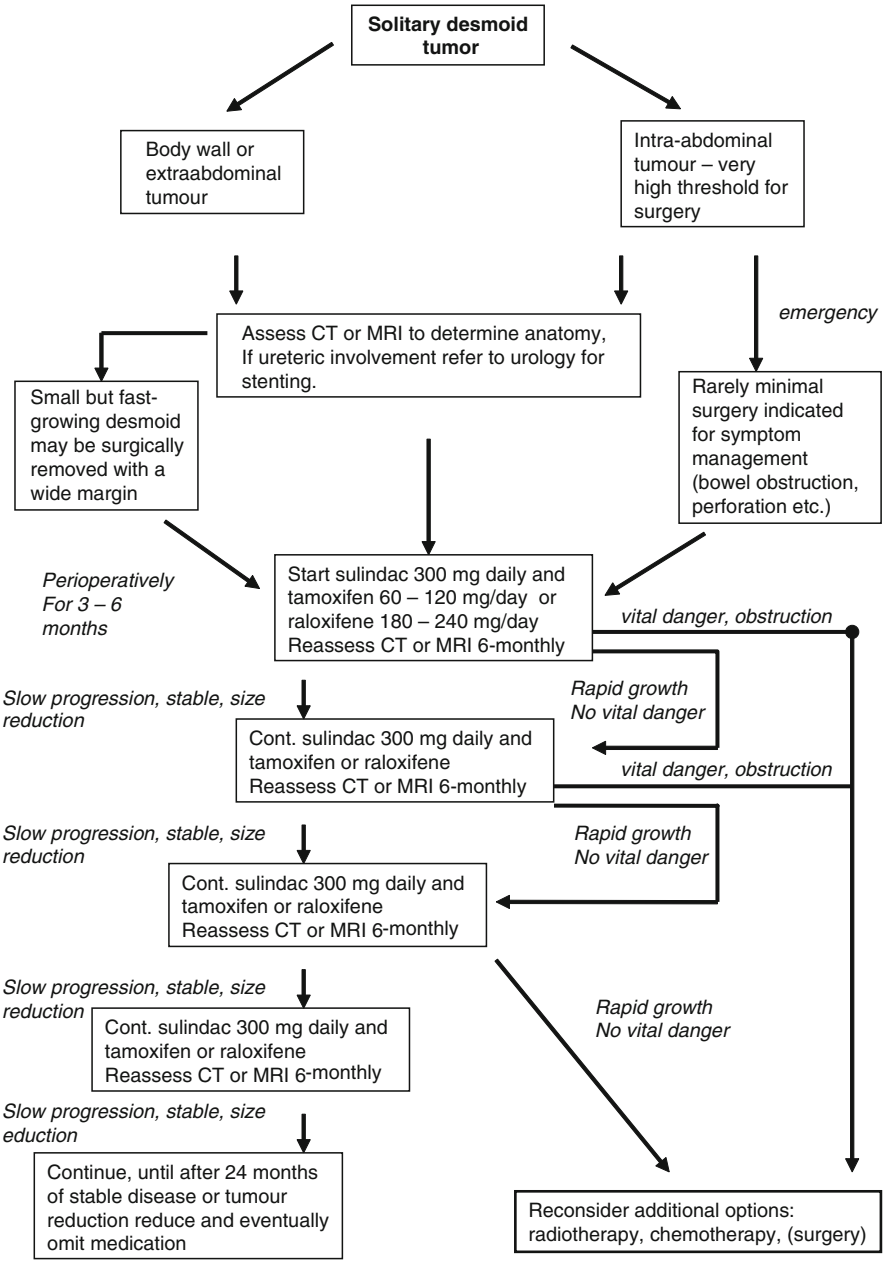

Fig. 13.2 Proposed algorithm for treatment of desmoids tumour

In summary, FAP-associated desmoids tend to arise in the abdomen or abdominal wall. CT scanning gives the best information on tumour anatomy whilst T2-weighted MRI characterizes the tumour best. Treatment may simply consist of observation. Otherwise, usual first-line therapy is with sulindac with or without an anti-oestrogen. Cytotoxic chemotherapy is an option in unresectable tumours. Surgery is a reasonable first-line treatment in abdominal wall tumours but is risky for intra-abdominal tumours and may necessitate massive small bowel resection (Fig. 13.2).

Take Home Message

- FAP-associated desmoids tend to arise in the abdomen or abdominal wall.
- MRI may allow classification of biological behaviour.
- Non-surgically treated desmoids respond better to medical therapy.
- Surgery for all desmoids leads to a high recurrence rate (conservative estimate 50%).
- Surgery and radiotherapy for desmoid tumours may cause mortality or substantial life-long morbidity.
- Aggressive growth pattern may best be identified via classification scores.
- Less aggressive approaches have a high success rate and a low recurrence rate.
- For some desmoids (DES 0 and 1), observation only is justified.
- The best medical approach for treatment of desmoid tumours must yet be delineated – however, there is substantial evidence for a high response rate to high-dose anti-oestrogen and NSAID therapy.

As a final conclusion, there is desperate need for international studies in order to better understand aetiology and treatment response in this enigmatic tumour.

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Chapter 14
Attenuated Familial Adenomatous Polyposis: Diagnosis, Management, and Future Prognosis

Thérèse M.F. Tuohy and Randall W. Burt

Abstract Attenuated familial adenomatous polyposis is a variant of familial adenomatous polyposis (FAP) in which patients present with 99 or fewer cumulative polyps in the colon and/or rectum, with a tendency toward more proximal colonic polyps [1, 2]. The average age of adenoma development and of colon cancer occurrence is clearly older than in typical FAP. However, there is a wide variation in the age of onset of these lesions and a wide variation in the numbers of adenomas in individuals within attenuated FAP families, with some individuals presenting with more than 100 lifetime polyps. Therefore, it is difficult and often impossible to distinguish attenuated FAP from typical FAP in single individuals. The most suggestive cases initially are patients over the age of 50 who exhibit 50–100 adenomas. Younger patients with fewer adenomas and older patients with greater than 100 adenomas are difficult to categorize. This means that examination of multiple family members is often needed to distinguish FAP and attenuated FAP in a family. Genetic testing is now also helpful in this regard, as mutations giving rise to attenuated FAP preferentially occur in localized regions of the *APC* gene.

Keywords Attenuated • FAP • Familial polyposis • Polyposis • Colon cancer

14.1 Clinical Presentation

It is notable that in some of the better-studied FAP kindreds, the variation in colonic polyp number can be striking, with some family members exhibiting few adenomas, even at older ages, while others may be found to have hundreds (but never thousands) of adenomatous polyps [3–5]. It is generally noted that the lifetime

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colon cancer risk is lower than that observed in classic FAP; ~70% compared with virtually 100% for classic FAP, with an approximately 15-year delay in average age of cancer diagnosis and mortality [1, 6, 7].

Fewer data are available for lifetime risks for extracolonic FAP-associated cancers. It appears that the risk of gastric fundic gland and duodenal/ampullary cancers are similar to that in classic FAP kindreds – of the order of 3–5% [1, 2]. The risk for developing fundic gland polyps is ≥50% and for developing duodenal adenomas may be as high as high as 90%. For *APC* carriers, these risks appear to vary with mutation location. Proximal *APC* mutations appear to be associated with a higher risk for upper gastrointestinal polyps and fewer adenomas. The reverse appears to be true for distal *APC* mutations. Risks for other FAP-associated cancers in attenuated FAP patients are also not well documented [2, 8–10]; there is no evidence that the attenuation of the colonic phenotype is associated with a concomitant attenuation of the risk for extracolonic cancers. The risks of developing other extracolonic manifestations, which have independently been shown to have phenotype–genotype correlations, appear to track with the site of the mutation in attenuated FAP families, similar to their observation in typical FAP families, rather than tracking with the penetrance of the colonic phenotype [11–13]. These include desmoids, osteomas, and CHRPE (congenital hypertrophy of the retinal pigmented epithelium). The lack of clear evidence to the contrary has lead most practitioners to err on the side of caution in screening and management recommendations (see below):

Clinical features of attenuated FAP

Clinical finding	Frequency and other details in attenuated FAP
Colonic polyposis	Few to hundreds of polyps, but extremely variable in individual members of attenuated FAP families
Colonic polyp distribution	Adenomas frequently more proximal in colonic locations compared to equal distribution observed in typical FAP
Age of colonic polyp onset	10–15 years later than in typical FAP
Lifetime risk of colon cancer	Approaches 70%
Average age of colon cancer occurrence	10–15 years later than in typical FAP, thus approximately 50–55 years.
Fundic gland polyposis	Risk of occurrence and polyp numbers are similar to typical FAP, but occurrence varies somewhat with mutation location, i.e., persons with proximal *APC* gene mutations tend to have fundic gland polyposis more commonly
Duodenal adenoma formation	Same as typical FAP
Osteomas	Observed, especially if *APC* mutation in the distal portion of the gene, frequency uncertain
Extracolonic malignancies	Gastric and duodenal malignancies occur with the same frequency as in typical FAP
	Other sites, i.e., thyroid, CNS, pancreatic, hepatoblastoma, and adrenal, uncertain

As the underlying molecular etiology of the inherited predisposition to polyposis and cancer becomes clearer, the necessity for more precision in the clinical

nomenclature will help stratify clinical risks and management in these patients. The terms FAP and attenuated FAP were historically used as clinical descriptors for patients with variants of adenomatous polyposis. As our molecular and clinical understanding has progressed, they are increasingly reserved for two scenarios. The first is for patients from families with a suspected or established autosomal dominant inheritance pattern of polyposis. The second is for index cases without a family history in which a deleterious mutation or genetic linkage has been found in the *APC* gene. MAP, *MUTYH*-associated polyposis, is used to refer to polyposis patients in whom a deleterious mutation has been found in *MUTYH*. Patients with multiple colorectal adenomas (MCRA), in whom neither *APC* nor *MUTYH* mutations are detected, are a heterogeneous group, both clinically and genetically. Some may have occult mutations in *APC*, or other known genes, beyond the current limits of standard detection methods, while others may have mutations in as-yet unknown genes [14, 15]. Some may represent somatic (or mosaic) mutations [16, 17] while others may be phenocopies, due to non-genetic causes.

14.2 Clinical Management

Because of the delay and moderation in symptoms compared with FAP, attenuated FAP patients may fail to be differentiated from relatively common cases of sporadic "polyp formers" in the general population. In the largest attenuated FAP kindred studied to date, ~21% of the genetically ascertained patients between the ages of 30 and 79 presented with six or fewer polyps at the time of their initial colonoscopy and 7% with *none* [1]. Combined with the fact that extracolonic manifestations such as fundic gland polyps are usually asymptomatic [6], this syndrome may be significantly underrecognized and underdiagnosed. Adherence to population-based guidelines, such as those of the American Gastroenterological Association, the American College of Gastroenterology or the American Cancer Society, would detect many of these cases if initiated beginning at the age of 50. However, these guidelines, which allow for sigmoidoscopy in low-risk populations, may fail to detect the predominantly proximal polyps in attenuated FAP patients due to the more frequent proximal location of these lesions.

Current management in established attenuated FAP kindreds can be based on the results of genetic testing; only those individuals who inherit the family mutation need to engage in a protocol of regular colonoscopic screening. Screening should start around the time of initial genetic testing, usually in the late teens. In the absence of other contributory factors, mutation-negative individuals in families with a known mutation may safely follow population guidelines for colon cancer screening. First- and second-degree relatives of patients in families with an apparent Mendelian inheritance pattern, but without a known mutation must be managed as at-risk individuals.

Recommended screening guidelines for attenuated FAP and at-risk patients

GI location	Screening	Age to start	Interval
Colon[a]	Colonoscopy	18–20 years (earlier if symptoms occur or depending on individual family history)	Every 2–3 years
Duodenum, Stomach	Upper GI exam with side-viewing scope	20–25 years	Every 1–3 years, depending on polyp number, size, and histology
Small bowel	Small bowel imaging	If large adenomas are found in duodenum or symptoms referable to the small bowel occur	As needed
Rectal screening after colectomy with ileo-rectal anastamosis[a]	Sigmoidoscopy	Within 6–12 months following surgery	Every 1–3 years, depending on polyp number

[a]Individuals who have had colon surgery should be managed appropriately for remaining colon

As the polyp load in the rectum is usually light, recent guidelines suggest that the surgical management option be IRA (ileo-rectal anastomosis) for attenuated FAP patients [18], in order to balance quality of life issues with post-surgical polyp and cancer risks. However, classic FAP patients generally develop higher numbers of polyps, and have associated higher rectal cancer risks. Therefore, the more conservative option of total proctocolectomy with ileal pouch–anal anastomosis (IPAA) or restorative proctocolectomy has been preferred, depending on the severity of rectal polyp involvement. Continued rectal screening, particularly in IRA-treated patients is critical, as demonstrated by the occasional development of subsequent rectal cancer, even in patients taking sulindac for the suppression of polyp growth [19, 20]. Patients with attenuated FAP also frequently develop rectal segment adenomas and even rectal cancer and thus likewise require close rectal screening [1].

14.3 Molecular Genetics

In the clinical setting, germline mutations in *APC* are found in 60–80% of patients who present with a classic FAP phenotype [21], and typically only in 20–30% of patients with the milder or attenuated phenotype [22]. One registry found that the majority, 72%, of kindreds with the attenuated phenotype were found to have either *APC* or *MUTYH* mutations [7]. Nonetheless, the overall success of finding *APC* mutations in typical FAP compared to attenuated FAP is 60–80%

versus 20–30%, respectively. Several factors may contribute to the lower rate of finding responsible mutations in patients with an attenuated phenotype. Patients with multiple adenomas may have MAP, while others may be sporadic, environmentally related, or arise from mutations in other known, or yet unidentified genes. Some patients with lower numbers of adenomas also exhibit other histological types of polyps, and are actually more correctly classified as mixed polyposis rather than attenuated FAP. Mixed polyposis may therefore be a distinct diagnosis, attributable to causes other than *APC* mutation.

It is possible that some patients who present with attenuated FAP are mosaic for *APC* gene mutations. In this situation, only some colonic stem cells have developed a causative mutation, so the polyp number is lower. In other or some cases, the mutation may be below the level of detection in peripheral blood [16, 17]. Some of these patients may be at risk to pass the mutation on to their children, if the mutation occurred at a point in embryonic development that preceded the development of the reproductive cells. If the mutation developed later in embryonic development, after the commitment of the germ-line to their reproductive fate, then the mutation will not be transmissible to the patient's children. However, there is no way to distinguish clinically between these scenarios.

In other cases, it is possible that undetected mutations deep within introns of *APC*, in areas not included in standard commercially available sequence analysis, alter the normal splice profile of *APC* gene expression, generating out-of-frame transcripts. This has been demonstrated for *APC* as well as other conditions [23–25]. *APC* mRNA instability resulting from spurious generation of in-frame stop-codons, leading to nonsense-mediated decay of those aberrant transcripts, may account for some attenuated FAP cases. This may explain some of the cases reported for the 3′ mutations for which reduced, if any, mRNA or truncated proteins could be detected from the affected alleles [26, 27]. Recent evidence suggests that partial deficiency in expression of one allele may be sufficient to lead to a delayed phenotype, as evidenced by loss of heterozygosity (LOH) of the fully expressed allele in tumors [14, 28]. Total or partial haploinsufficiency may also be caused by promoter mutation [29]. Finally, it is possible that some clinical diagnoses of FAP and presumably attenuated FAP are due to mutations in other genes [14], including *MUTYH* and *OGG1* [7, 30, 31].

14.4 Genotype–Phenotype Correlations

With respect to *APC*, attenuated FAP has previously been seen as a subset of FAP, in which the mutations cluster in a number of 'hotspots'. These are, to a first approximation,

- At the 5′ end of the gene, upstream of codon 157,
- At the 3′ end of the gene, downstream of codon 1595, and
- In exon 9A, the alternatively spliced exon proximal to, and contiguous with, exon 9.

However, the exact boundaries appear to be somewhat undefined. For example, at the 5′ end of the gene, in some cases, c.505_509delATAGA appears to predispose to classic FAP [32], whereas other studies have shown that this mutation can also be associated with an attenuated phenotype [33]. Mutations leading to both FAP and attenuated FAP have been reported at the 3′ end of the gene [4, 34]. Other cases have accrued in the literature, on a case by case basis, detailing attenuated FAP patients and families with mutations in a variety of other locations, including exon 6, exon 9, exon 13, exon 14, and exon 15 [23, 33, 35–37]. These mutations, when examined closely (see below) have the common theme of incomplete penetrance at the protein level; the resulting APC protein appears to retain significant function. Cumulatively, these data have generated a picture of *APC* with two major components: qualitatively, the core function is encoded between exon 4 and the distal portion of exon 15, and quantitatively, it appears that even a modest decrease from normal levels of gene expression can lead to an attenuated FAP phenotype.

Within common mutation types, internal and cytogenetic deletions, frameshift and termination codon mutations, splice-site mutations, etc., there are examples of mutations in *APC* that might, at first glance, have been expected to confer a classic FAP phenotype. The myriad exceptions to the rules offer multiple insights into the vagaries and subtleties of gene expression. On-going research, especially with the advantage of extensive kindreds and ever-more detailed molecular readouts of normal and pathologically affected tissues promises to dissect the functional loss incurred by attenuated FAP patients.

14.5 Deletions

Interestingly, the mutation at codon 1860–1862 (c.5580_5583delTTCT) in a family resulted in a null allele for *APC*, as demonstrated by Western blot analysis, and was found to be associated with FAP [27]. This mutation falls within the classic 3′ attenuated domain, which has previously been postulated to be partially dispensable for cellular activity [14]. However, it may function by destabilizing the mRNA, then perhaps effectively falling into the same category as others reported later [38, 39] which may owe their attenuated phenotype to partial function of the mutant alleles.

Cytogenetic deletions are generally associated with a classic FAP phenotype. They have also been occasionally associated with attenuated phenotypes. This cannot be attributed to *partial* insufficiency of the defective allele [40, 41]. It may be important to note, however, that in these cases, extensive interfamilial analysis was not available, and the attenuated phenotype may have been due in large part to an unusually ‘protective’ normal *APC* allele, inherited from the other parent. The existence of one or more hypothetical “super-alleles” (normal population variants that are in the top ~5th percentile with respect to expression levels, half-life in the cell, or functional ability in physiological pathway(s)) may ultimately contribute to our understanding of these mutant anomalies. Such variants of *APC* [40] may explain whole or partial gene deletions that have been reported with a relatively mild, attenuated, rather than

classic FAP phenotype [23, 42]. They may also explain the variable phenotype associated with other deletions that are observed as normal low-level splice variants in the general population, such as the alternative splicing of exon 14 [23, 43].

14.6 Splicing Mutations

Recent analysis of silent mutations in *APC* as well as other genes has shown that nucleotide substitutions that fortuitously change the context of the sequence in which they are embedded, to more closely matched consensus sequences for exonic splicing enhancers (ESEs) or splice donor/acceptor signals, may lead to aberrant splicing events. The efficiency of these aberrant splicing events is directly related to the level of homology to evolutionarily conserved *bona fide* splice signals [44]. For example, S457X encoded by exon 10 appears be associated with an attenuated phenotype [45], although the mutation would predict a classic phenotype [46]. Analysis using the software package https://splice.cmh.edu/ [35, 47] suggested that this anomaly might be at least partly explained if the underlying DNA mutations were c.1370C>A (Serine to UAA stop codon). This was reported for the FAP kindred, while c.1370C>G (Serine to UGA stop codon) was reported for the attenuated kindred (not published). c.1370C>A reduces the theoretical binding capacity of SRp40 by 73.3%, while c.1370C>G reduces it by 42.8%.

In exon 6, c.697C>T (encoding Q233X) has been reported to be associated with a delayed phenotype [48], possibly due to altered ESE signaling that allows for the generation of compensatory alternative splice forms as predicted by http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home [49, 50]. This mechanism has also been implicated in the case of c.847C>T (encoding R283X) in exon 8 [51] which was both predicted to improve the consensus recognition sequence for the SRp40 splicing factor and experimentally shown to generate exon 8-deficient splice forms.

Mutations in exon 9A, (c.1087_1088insA) [52], can be alternatively spliced, leaving an effectively full-length, or almost full-length functional protein that can also give rise to attenuated FAP. The notion that dosage is a critical feature in the molecular dichotomy between FAP and attenuated FAP phenotypes is further underscored by contrasting mutations reported at the exon 9 splice donor, as depicted in Fig. 14.1.

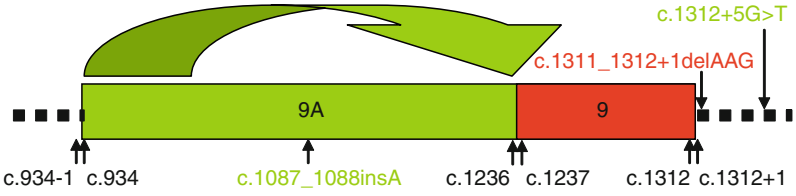

Fig. 14.1 Schematic representation of exons 9 and 9A of *APC*. Green regions indicate the alternatively spliced exon and locations where mutations tend to be associated with attenuated FAP, *red* with FAP. The *large green arrow* indicates the normal alternative splice form that omits exon 9A, and the *black vertical arrows* indicate cDNA nucleotide positions of exon–intron boundaries and mutation positions discussed in the text

For example, c.1311_1312+1delAAG, which ablates the splice site at the 3′ junction of exon 9, results in FAP [37]. However, c.1312+5G>T, just four nucleotides further downstream, which both theoretically and experimentally reduces its efficiency, results in attenuated FAP [53].

14.7 Modifiers

Considerable inter- and intrafamilial variation has been reported not only in the polyp number, but also in the overall expressivity of the condition in both FAP and attenuated FAP patients. Because the numbers of polyps in attenuated FAP patients is lower, the relative level of variation appears higher. Whether this is stochastic fluctuation, due to background noise or true variation, due to the innate properties of the mutant *APC* alleles involved, or due to unlinked modifiers remains to be seen. *APC* may be a modifier of its own function. This could be mediated at the level of influencing the nature of the selection pressure on the molecular properties of the second hit presumed to be a prerequisite for polyp initiation [54, 55]. Alternatively, a "third hit", in the form of a secondary mutation to an attenuated allele, to completely ablate its function, could influence the rate at which polyps and extracolonic features present [41]. Individual normal population variant alleles of *APC* were also considered as candidate modifiers of FAP phenotypic variation and found not to account for significant variation [41]. However, as acknowledged by the authors, the families examined were small, and an approach designed to identify potential *APC* modifying alleles by comparing phenotypes and *APC* haplotypes of siblings among several branches of larger kindreds might yield a different answer.

Analysis of potential unlinked modifiers identified *NAT1* and *NAT2* as modifiers of *APC*, accounting for approximately a twofold variation in polyp number between carriers and noncarriers [56]. Preliminary experiments in a mouse model of FAP show an interdependency between ornithine decarboxylase (ODC) expression levels and polyp count [57] while a polymorphism of the *ODC* gene in the human population appears to have a protective effect against adenoma formation [57]. Whether this is further found to modify polyp number in attenuated FAP kindreds remains to be seen. Other potential modifiers include polymorphic variants of drug-metabolizing drugs, for example, COX2 inhibitors, which have been examined in colon cancer and population-based cohorts [58–60], but not yet in FAP or attenuated FAP populations or kindreds.

Fine mapping of genetic modifiers of polyp number in the *Min* mouse has yielded three loci to date, *Mom1, Mom2, and Mom7* [61–64]. These are strain-specific heritable modulators of polyp number that segregate independently of the *Min* mutation in successive backcrosses between lines of inbred mice. *Mom1* was shown to be due to a frameshift mutation of *Pla2g2a*, a secreted form of phospholipase IIA [65]. However, in humans, no deleterious mutations were found in either PLA2G2A or either of its linked orthologs, PLA2G2C and PLA2G5, that correlated with variation in polyp number of the c.426_427delAT attenuated FAP kindred [66, 67]. *Mom2* was shown to be due to a 4-bp duplication within exon 3 of *Atp5a1,*

the gene for the α subunit of ATP synthase [61]. Whether variants of this gene modify penetrance of *APC* mutations in humans has not yet been reported. *Mom3* appears to be a complex variant that maps close to *APC* and is also subject to additional influences due to pregnancy [68, 69]. *Mom7* was recently reported to map to a 7.4 Mb interval proximal to *APC* on chromosome 18 [63], which is syntenic to portions of human chromosomes 10, 18, 2, and 5.

As work on these models progresses, it is likely that variants, if not deleterious mutations will be found for most if not all homologs of the mouse modifiers. These variants may contribute to variation in polyp number within families with known mutations. Coinheritance of common polymorphic variants of different genes may also help to explain the multifactorial nature of familial colon cancer risk in the general population [70].

14.8 Outliers

While most mutations identified upstream of codon 157 in exon 4 have been associated with an attenuated FAP phenotype, there are some exceptions to this rule. Three generations of a family of FAP patients have been described with mutations in exon 3 (c.348_352delTTCAT) [71], as well as an FAP patient with an in-frame stop-codon mutation (c.376G>T) also in exon 3 [4]. In the absence of undetected linked second mutations, these cases are intriguing, as it is unlikely that unlinked modifiers would have been coinherited in all patients. This leaves the possibility of unusual secondary structure, transiently generated and "frozen" at the site of translational termination, which is incompatible with continued ribosome tracking, if downstream reinitiation at codon 184 is the unifying theme that explains the attenuated phenotype of the 5′ mutations in *APC* [72]. Most, although not all, mutations at the 5′ end of the gene are upstream of an in-frame potential internal initiation codon in exon 5. If this were used as a reinitiation codon after translation termination due to either in-frame or out-of-frame mutations, it would allow almost full-length APC protein to be produced from the distal transcript. Interestingly, the first four exons of *APC* are among those found to be alternatively spliced in both normal cultured lymphocytes and cancer cell lines [73], suggesting their dispensability for at least some normal cellular function. Conversely, two attenuated FAP families with mutations downstream of exon 157 [74] and an attenuated FAP family with a frameshift mutation in the beginning of exon 15 (c.3185_3186delAA) [75] which are not easily explained by any of the above models, suggest that still other mechanisms contribute to the control of expression of *APC*.

14.9 Summary

The diversity of the molecular basis for attenuated FAP appears to be richer than previously recognized. The unifying theme, if there is one, may be that any mechanism that compromises the expression level of a functional APC protein from the

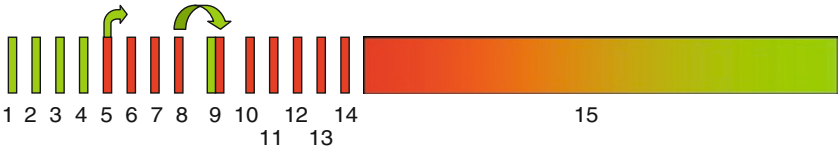

Fig. 14.2 Schematic representation of exons 1–15 of *APC*. *Green regions* indicate exon locations where mutations tend to be associated with attenuated FAP, *red* with FAP. The *arrows* indicate internal translation initiation and alternative splicing sites whose use could lead to the production of a core APC protein with partial function

mutant allele may be sufficient to predispose carriers to attenuated FAP. It appears that colon and other predisposed tissues are sensitive to the levels of APC in the cells. Not only is haploinsufficiency (due for example to loss or major disruption of gene function) a challenge that results in a high rate of downstream genetic disregulation, but even more subtle reductions in the expression levels of one allele may eventually lead to a clinical presentation [76]. The threshold level of normal function remains to be determined. This will probably not be an absolute level, but a range, depending on an array of environmental and genetic cofactors.

The range of the likely mutational spectrum for attenuated FAP, summarized schematically in Fig. 14.2, now includes examples of all of the following:

- 5′ of codon 157
- 3′ of codon 1595
- Alternatively spliced exon 9A
- mRNA insufficiency due to promoter primary or epigenetic mutations
- mRNA insufficiency due to cryptic splice mutations that result in nonsense mediated decay
- Cryptic splice mutations that generate an alternative transcript – any intron
- Cryptic splice mutations that arise from coding mutations, for example, exon 13.

14.10 Future Directions

Currently, the greatest challenges to differential diagnosis and management of attenuated FAP are (1) the relatively poor rate of detection of genetic mutations and (2) the classification of newly presenting patients and families. Profiling of tumors, from a cohort of *APC/MUTYH*/HNPCC mutation-negative MCRA patients, has shown that a molecular signature of somatic secondary mutations accumulated in the tumors reveals a profile distinct from that shown by MAP-associated tumors, and similar to that of sporadic, noninherited tumors [15]. Interestingly, these MCRA patients did not develop microadenomas, as FAP, attenuated FAP, and MAP patients do, suggesting that their genetic predisposition may be attributable to as-yet unidentified genes that contribute to progression rather than initiation of adenomas. Other techniques, aimed at a different level of profiling of the phenotype–genotype correlation for subclassification of patients, rather than pursuing

the ever more elusive single-gene mutation, may ironically, eventually allow for more customized and accurate patient management than our current system.

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Part V
Clinical Science: Hereditary Nonpolyposis Colorectal Cancer

Chapter 15
An Overview of the Lynch Syndrome (Hereditary Non-polyposis Colorectal Cancer)

Hans F.A. Vasen and J.C.H. Hardwick

Abstract Environmental factors play a dominant role in the etiology of most colorectal cancers. However, in about 5% of all cases, CRC is associated with a highly penetrant dominant syndrome. The most common of these is Lynch syndrome (hereditary non-polyposis colorectal cancer; HNPCC). It is characterized by the development of colorectal cancer, endometrial cancer and various other cancers and is caused by a mutation in one of the mismatch repair (MMR) genes: *MLH1, MSH2, MSH6* or *PMS2*. The MMR-defect leads to instability at microsatellites of tumour-DNA (microsatellite instability (MSI)) which can be found in >90% of CRC associated with Lynch syndrome. Currently, mainly clinical criteria (Bethesda criteria) are used to select patients with CRC for molecular genetic (MSI-analysis) and/or immunohistochemical analysis of the tumour and those with evidence of MSI or loss of MMR expression are offered mutation analysis. Because, there is increasing evidence that MSI/IHC is an important prognostic factor and may predict the response to chemotherapy, these tests might in future be performed on a much larger scale, if not in all CRC cases.

Identification of Lynch syndrome families is important as it allows to offer preventative measures. Many studies have shown that colonoscopic surveillance leads to a substantial reduction of the risk of CRC and also reduction of the mortality associated with CRC. Also surveillance for endometrial cancer may lead to detection of premalignant lesions and early cancers.

Knowledge on the effectiveness of surveillance for the other LS-associated cancer is limited.

The life time risk of developing one of associated cancers (stomach, ureter, renal pelvis, small bowel, the bile ducts and tumors of the brain) is relatively low

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(less than 15%) and may be associated with the underlying MMR defect. In the decision making on which surveillance protocol should be recommended, a reasonable approach might be to first discuss all the various cancer risks with the patient, then discuss which screenings are indicated scientifically.

In this chapter, the clinical features, diagnostic criteria and management of the syndrome will be discussed in detail.

Keywords Overview • Lynch syndrome • Hereditary • Non-polyposis • Colorectal cancer • HNPCC

15.1 Introduction

The first family with dominant clustering of cancer of the stomach, colorectum and the endometrium was described by Alfred Warthin in 1913 [1]. Henry Lynch and Ann Krush updated the same family almost 60 years later [2]. In the next 25 years, Henry Lynch was almost the only investigator who continued to describe the syndrome in numerous reports and continued to ask attention for the syndrome. In the mid 1980s several groups of European investigators realised the importance of these studies and started a search for Lynch syndrome (hereditary non-polyposis colorectal cancer (HNPCC)) families in their respective countries [3–5]. In 1990, an International Collaborative Group was set up to establish criteria for the Lynch syndrome and to promote international collaborative studies [6]. Due to this worldwide collaboration, the genes responsible for the syndrome were identified in a relatively short time. Lynch syndrome is the most common dominantly inherited colorectal cancer syndrome responsible for 3–5% of all colorectal cancer cases. It is characterized by the development of colorectal, endometrial and various other cancers at an early age [7]. The syndrome is due to a mutation in one of the following DNA-mismatch repair genes: *MSH2*, *MLH1*, *MSH6* and *PMS2* [8]. A defect in these genes leads to multiple errors in repetitive DNA sequences (microsatellites) throughout the genome of tumours. This form of genomic instability is called microsatellite instability (MSI) and is the hallmark of the Lynch syndrome. Various names for Lynch syndrome have been used in the past century, including cancer family syndrome, hereditary non-polyposis colorectal cancer (HNPCC), colorectal endometrium cancer syndrome and Lynch syndrome type I and II [9, 10]. The International collaborative group agreed in Amsterdam in 1990 upon the name HNPCC because at that time the syndrome was unknown to most doctors. This name clarified that the syndrome described an inherited form of CRC. The appropriateness of the name was discussed again at an international meeting in Bethesda in 2004. Most participants considered the term HNPCC to be inappropriate, since the syndrome is also associated with many other tumours. It was proposed that the name *Lynch syndrome* (*LS*) should be reintroduced and that this name should be reserved for families with strong evidence of mismatch repair deficiency, for example, by the presence of an MMR defect or the presence of MSI in tumours [11].

The identification of individuals predisposed to colorectal cancer is important, as it makes it possible to target effective preventative measures. Moreover, it is important to differentiate the LS from other hereditary/familial colorectal cancer syndromes because the surveillance programme and treatment of this syndrome differ from that of the other syndromes. In this chapter, the clinical features, diagnostic criteria and management of the syndrome will be discussed.

15.2 Clinical Features

LS is characterized by several unique clinical and pathological features. Knowledge of the specific features is crucial for the identification of the syndrome. Table 15.1 shows a list of the most important characteristics.

15.2.1 Autosomal Dominant Inheritance

One of the cardinal features of LS is autosomal dominant inheritance. In a pedigree with classical LS, half of the relatives in successive generations have colorectal, endometrial or another cancer. In contrast with familial adenomatous polyposis in which about one-third of the cases are caused by a de novo *APC*-gene mutation, LS families based on a de novo mismatch repair gene-mutation are rarely reported [12, 13]. In such families, the parents and siblings of the patient do not develop the disease. Due to the high penetrance of the syndrome associated with *MLH1*, *MSH2* and *MSH6* mutations, family members in successive generations are affected and skipping of generations is rarely observed. That is the reason that colonoscopic surveillance in LS families without a known mutation is usually recommended only in first-degree and not in second-degree relatives of affected family members. On the other hand, skipping of generations is frequently observed in families with a deleterious *PMS2* mutation. In families with such mutations, the inheritance appears to be autosomal dominant with a variable penetrance.

Table 15.1 Cardinal features of the Lynch syndrome

Autosomal dominant inheritance
Associated cancers: cancer of colorectum, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, skin (sebaceous adenoma)
Development of cancer at an early age
Development of multiple cancers
Features of colorectal cancer: predeliction for proximal colon, improved survival, multiple colorectal cancers, poorly differentiated tumours, tumour-infiltrating lymphocytes and Crohn's-like lymphoid reaction
Features of adenomas: the numbers vary from one to a few, increased proportion of adenomas with a villous growth pattern, high degree of dysplasia, rapid progression from adenoma to carcinoma
High frequency of microsatellite instability
Immunohistochemistry: loss of MLH1, MSH2, MSH6 and PMS2 protein expression

15.2.2 Cancers Associated with the Lynch Syndrome

A wide spectrum of cancers may be found in LS. The pattern of the cancer sites has changed over time. In the first family with the syndrome described by Warthin in the beginning of the twentieth century, gastric and endometrial cancer were the most common cancers, whereas in the generations of the same family described by Lynch in 1971, colorectal cancer was the most frequent tumour [1, 2]. The variation of the pattern of cancer over the years reflects the change in incidence of cancers in the population over the same period of time. Also current differences in the expression of LS between families from Western countries compared to families from the Far East reflect the variation in incidence of cancers in the respective populations [14]. These observations suggest that even in this hereditary condition, environmental factors play an important role in the carcinogenesis.

For the decision-making on which surveillance programme should be recommended, we need to know which tumour belongs to the tumourspectrum of LS. There is no doubt that in addition to cancer of the colorectum and endometrium, cancer of the small bowel, stomach, urinary tract (pelvis and ureter), ovaries, brain, biliary tract and sebaceous tumours are associated with the syndrome. For most of these cancer sites, studies have shown that the observed/expected ratio is increased [15–19]. However, there is still uncertainty whether also cancer of the breast and prostate belong to the LS tumourspectrum [20–22].

Although most studies have not reported an increased incidence of breast or prostate tumours in LS families compared to the general population, studies have reported breast or prostate cancers showing MSI in patients from LS families. However, the question arises whether the finding of MSI in a specific tumour of a patient from an LS family proves that this tumour is associated with the syndrome. It might be hypothesized that the mismatch repair gene mutation causing MSI plays a role in the progression of the cancer, but that the mutation is not involved in the initiation of the tumour (which should have led to an increased incidence).

The genetic basis of the tumourspectrum of LS is incompletely understood. The mutations driven by MMR deficiency may affect important growth-regulating genes, especially those containing repeat sequences. These mutations show considerable tissue specificity. For example, mutations affecting repeat tracks within *TGFbetaRII*, *BAX* and *TCF4* are strongly selected in gastrointestinal malignancies but not in endometrial cancer. Such tissue-specific selection may therefore provide one possible explanation for the LS-associated tumourspectrum.

Decisions as to whether surveillance should be advised for a specific type of cancer should be based on the age-specific cancer risk and the availability of sensitive and specific screening tools.

15.2.3 Cancer Risk and Geno/Phenotype Correlations

The lifetime risk of developing colorectal cancer in carriers of a mismatch repair defect reported in the literature varies from 25 to 80% [16, 23–27]. The risk

Table 15.2 Lifetime risk of developing various types of cancer in the Lynch syndrome

Colorectal (men)	28–75%
Colorectal (women)	24–52%
Endometrial	27–71%
Ovarian	3–13%
Gastric	2–13%
Urinary tract	1–12%
Brain	1–4%
Bile duct/gallbladder	2%
Small bowel	4–7%

depends on sex and the type of mismatch repair gene involved. Most reports on the risk of this cancer are biased as the families were selected on the basis of clustering of colorectal cancer. Recently, Sining Chen et al. performed a meta-analysis using only the population-based studies and one additional analysis that adjusted for ascertainment [25, 27–30]. They reported a cumulative risk of CRC at 70 years of age for male *MLH1* and *MSH2* carriers of 55% and for female *MLH1* and *MSH2* carriers of 35%. The risk of developing CRC at 70 years of age for carriers of male and female *MSH6* carriers was similar, that is, 35%.

The risk of developing endometrial cancer is reported to be 30–60%. The risk of other cancers associated with Lynch syndrome is less than 10–15%. (Table 15.2) There are a few reports that suggest that extracolonic cancers are more frequent in families with an *MSH2*-mutation than in families with an *MLH1*-mutation [24, 31]. Families that harbour *MSH6*-mutations are characterized by development of colorectal cancer at a more advanced age and a higher risk of developing endometrial cancer. Families associated with *PMS2*-mutations are very rare. In a recent report on seven families with an identified *PMS*-mutation, three fulfilled the Amsterdam criteria (see below) [32]. The pattern of inheritance was autosomal dominant with variable expression and a milder phenotype compared to families with an *MLH1* or *MSH2*-mutation.

15.2.4 Modifier Genes

Although all of the MMR gene mutation carriers are predisposed to developing the cancers that are characteristic of LS, there is considerable variability in their age of onset. This variability is probably due to a combination of genetic and environmental factors. During the last decade, a large numbers of studies have been performed to evaluate the effect of polymorphisms in genes that play a role in carcinogenesis on the age of onset of CRC in LS [33–50]. These genes include those that influence the metabolism of known carcinogens (*NAT2*, *GSTM1*, *GSTT*), genes that affect the cell cycle (*Cyclin D*), and genes that induce apoptosis (*P53*). The results are summarized in Table 15.3. A significant association was found between the age of onset of CRC and *GSTTM* and *GSTT*-polymorphisms. Moreover, a few single studies reported a positive association between the age of onset of CRC and *IGF1*, *RNASEL*, *CYP 17* and *DNMT3b*-polymorphisms. However, for the other polymorphisms, the findings were inconsistent. Explanations for these results may include geographic differences,

Table 15.3 The association between polymorphisms and age of onset of colorectal cancer in Lynch Syndrome

Polymorphism	Author/year	Nr carriers	Type mutation	Association
NAT 1 10+	Moiso 1998	182	*MLH1*	+
NAT2	Heinemann 1999	78	*MLH1/MSH2*	+
NAT2	Frazier 2001	86	*MLH1/MSH2* (*PMS2*)	+
NAT2	Pisterius 2006	226	*MLH1/MSH2*	–
Cyclin D1	Kong 2000	86	*MLH1/MSH2* (*PMS2*)	+
Cyclin D1	Bale 2001	146	*MLH1/MSH2*	–
Cyclin D1	Kruger 2006	406	*MLH1/MSH2*	–
GSTM1/GSTT1	Moiso 1998	182	*MLH1*	+
GSTM1/GSTT1	Felix 2006	129	*MLH1*	+
P53	Jones 2004	92	*MLH1/MSH2*	+
P53	Sotamaio 2005	193	?	–
P53	Kruger 2005	167	*MLH1/MSH2*	+
P53	Talseth 2006	220	?	–
MDM2	Sotamaio 2005	193	?	–
MDM2	Talseth 2006	220	?	–
ATM	Maillet 2000	67	*MLH1/MSH2*	+
RNASEL	Kruger 2005	251	*MLH1/MSH2*	+
IGF1	Zecevic 2006	121	*MLH1/MSH2*	+
CYP17	Campbell 2007	146	*MSH2*	+
COMT	Campbell 2007	146	*MSH2*	–
DNMT3b	Jones 2006	146	*MLH1/MSH2*	+

differences in methodology and various forms of bias of ascertainment. Further studies on large series of mutation carriers are needed to identify the polymorphisms that are most strongly associated. Hopefully, these polymorphisms may be helpful to identify high-risk individuals who require more intensive surveillance.

15.2.5 Early Age of Onset

All cancers related to the syndrome are characterized by an early age of onset. It has been suggested that the presence of a germ-line mutation in one of the mismatch repair genes at birth is responsible for the early age of onset of cancers in LS.

Previous studies have suggested that age at diagnosis of colorectal cancer decreases in successive generations of LS families (termed anticipation) [51]. Indeed, a study on 51 families with LS reported an increasing relative risk (RR) of colorectal cancer in successive generations [52]. However, adjusting the effect of generation for the secular trend resulted in non-significant RRs close to unity suggesting that the higher risk at younger ages in successive generations can be attributed to a secular trend in cancer rather than generation. A further study has also failed to find genetic anticipation [53]. The authors suggested that anticipation appears to reflect a birth cohort bias of ascertainment.

15.2.6 Occurrence of Multiple Tumours

One of the most characteristic features of LS is the occurrence of multiple tumours in a patient. In a series of 477 patients with colorectal cancer from families with a known mismatch repair gene mutation, 18% had a synchronous or metachronous colorectal cancer. In patients with a combination of colorectal cancer and LS-related cancer, microsatellite instability has been reported in almost 50% of the cases, and in up to 20%, an underlying mismatch repair defect may be detected [54]. Because of the increased risk of developing a colorectal cancer after a primary colorectal cancer, some authors recommended more extensive surgery, that is, subtotal colectomy for a patient with a primary colon tumour who belongs to a Lynch syndrome family [55].

15.2.7 Features of Colorectal Adenoma and Carcinoma

The adenoma–carcinoma sequence appears also to be applicable in LS. A recent study showed that carriers of a mismatch repair defect develop adenomas more frequently than controls [56]. The adenomas in carriers were found to be larger and a significantly higher proportions showed histologic features that are associated with a high risk of malignant degeneration, such as a high degree of dysplasia and the presence of more extensive villous architecture [56, 57]. Carcinomas in LS are predominantly located in the proximal colon. A recent study from Finland, showed that the risk of developing adenomas by age 60 was 68% in men and 48% in women. Half of the adenomas were located proximal to the splenic flexure. Table 15.4 shows a summary of the natural history of LS based on data from the Dutch Lynch syndrome registry. Carriers under surveillance develop their first adenoma at a mean age of 43 years and colorectal cancer at a mean age of age 46. These findings in combination with the observation that a relatively high proportion of patients develop colorectal cancer within three years after a clean colonoscopy suggest that the adenoma–carcinoma sequence is accelerated and that the progression from adenoma to carcinoma may take less than 5 years compared to 10–15 years in the case of sporadic colorectal cancer [58].

Most adenomas in carriers show MSI or absence of immunohistochemical staining of one of the mismatch repair proteins. MSI or immunohistochemical analysis may therefore be considered in young patients with large adenomas (e.g., >7 mm) with high-grade dysplasia. Several studies suggest that patients from LS families with

Table 15.4 Natural history of the Lynch syndrome

	Mean age (range) (years)
Appearance of colorectal adenomas[a]	43 (24–62)
Diagnosis of colorectal cancer[a]	46 (15–90)
Diagnosis of endometrial cancer	48 (24–78)
Death due to colorectal cancer	48 (19–91)

[a]Screen-detected

colorectal cancer survive longer than unselected colorectal cancer patients with tumours of the same stage [59]. However, because all studies reported so far are retrospective, it cannot be excluded that selection bias is responsible for this observation.

Colorectal cancer associated with LS tends to be diploid and is characterized by a significantly higher frequency of tumour infiltrating lymphocytes, mucinous histology, poor differentiation and Crohn's-like reaction.

15.2.8 Features of Endometrial Cancer

Endometrial cancer associated with LS is diagnosed approximately 10 years earlier than in the general population. The mean age at diagnosis of endometrial cancer in patients known at the Dutch Lynch syndrome registry is 48 years. The survival of patients with LS-associated-endometrial cancer appears not to be different from patients with unselected endometrial cancer [60]. The majority of endometrial cancers are of the endometroid type with diverse grading and staging [61]. Certain histopathologic features such as mucinous differentiation, solid-cribiform growth pattern, high grade and possible necrosis might suggest that a tumour is due to a mismatch repair defect. A recent study showed that endometrial cancer associated with the LS is characterized by poor differentiation, more frequent Crohn's-like lymphoid reaction, lymphangioinvasive growth and more tumour-infiltrating lymphocytes compared to sporadic endometrial cancer [62].

Loss of hMLH1 protein expression occurs in endometrial cancer associated with LS, but may also occur in 15–30% of sporadic cancers. Abrogation of MSH2 and/or MSH6 protein expression, especially at a young age is a more specific indicator for LS. Such loss of expression can already be found in the hyperplastic precursor lesions.

15.2.9 Features of Other Associated Cancers

15.2.9.1 Ovarian Cancer

A recent study revealed that compared to sporadic ovarian cancer, ovarian cancer associated with LS was diagnosed at an earlier stage [63]. The survival rate was not significantly different between patients with ovarian cancer associated with Lynch syndrome and the controls matched for age, stage and year of diagnosis. Also the pathology of LS-associated ovarian cancer appeared not to be different from sporadic ovarian cancer.

15.2.9.2 Gastric Cancers

Cancers of the stomach are mainly observed in the older generations of Lynch syndrome families reported in Western countries, but it is one of the most frequent extracolonic cancers in the current generations of LS families reported in the Far

East (Japan, South-Korea and China). A Finnish study reported clinical data on 45 patients with gastric cancer from 51 families [64]. The mean age at diagnosis was 56 years. Most tumours were of the intestinal type. Helicobacter Pylori infection was observed in only a minority of the cases. The 5-year survival rate was 15%.

15.2.9.3 Cancer of the Ureter/Pyelum

Relatively little information is available on cancers of the urinary tract associated with LS. A study reported seven cancers of the renal pelvis or the ureter in 50 families [18]. All were transitional cell carcinomas. The mean age at diagnosis was 58 years. The incidence of cancer of the bladder and kidney (excluding renal pelvis cancer) was not increased in these families.

15.2.9.4 Small Bowel

An international study among HNPCC Registries identified 42 individuals from 40 LS families who developed 42 primary and seven metachronous small bowel tumours [65]. There were 46 adenocarcinomas and three carcinoid tumours. The duodenum and jejunum was the most common site of primary small bowel tumours but tumours were also found in the ileum. The median age at diagnosis was 49 years. The small bowel was the first site of cancer in 24 patients (57%). The overall 5- and 10-year survival rates were 44% and 33%, respectively. A study from the German HNPCC consortium identified 32 cases of small bowel cancer in the HNPCC database [66]. The median age at diagnosis was 39 years. Fifty percent of the tumours were located in the duodenum. MSI was detected in 95% of the tumours and loss of mismatch repair protein expression in 89% of the cases. The pathology of the tumours was characterized by an expansive growth pattern of the tumour border and an intense intramural lymphocytic infiltrate. Based on these findings, the consortium proposed endoscopic surveillance of the duodenum (and stomach) in mutation carriers starting at age 30.

A recent Dutch study evaluated the cumulative incidence of SBC in a large series of proven mutation carriers [50]. A total of 28 patients of 1,496 (putative) mutation carriers were identified with SBC. The median age at diagnosis was 52 years (range: 23–69 years). The lifetime risk of developing SBC was 4.2%. The risk was lower in female carriers compared to male carriers but the difference was not significant. There was no difference in the risk of SBC between *MLH1* and *MSH2* carriers. SBC was not observed in *MSH6* carriers.

15.2.9.5 Brain

Studies have shown that brain tumours belong to the tumourspectrum of LS. Hamilton described two LS families with brain tumours (both glioblastoma) one harbouring a *PMS2* mutation and the other a *MLH1*-mutation [67]. We calculated a relative risk of six of developing such a tumour in patients with HNPCC and their

first-degree relatives [17]. The most frequent tumours observed at the Dutch Lynch syndrome registry were astrocytomas and oligodendrogliomas. Such tumours may occur at an early age as well as at an advanced age. In patients with brain tumours <20 years, the presence of bi-allelic mutations should be considered.

Brain tumours are an important cause of death in LS. An evaluation of the mortality in 140 families with an identified mutation revealed that brain tumours were the third most frequent cause of cancer deaths after colorectal cancer and endometrial cancer [68].

15.2.9.6 Skin Tumours

Skin lesions associated with LS syndrome are sebaceous tumours, including adenomas, epitheliomas and carcinomas, and keratoacanthomas. The combination of sebaceous neoplasms and an internal malignancy is known as the Muir–Torre syndrome [69]. This syndrome is most frequently found as a variant of LS. Physicians should consider MTS in patients presenting with a sebaceous neoplasm and immunohistochemical analysis of tumours for MSH2 and MLH1 expression can be used as screening test to identify LS.

15.2.10 Features of Individuals with Bi-allelic MMR-Mutations

About 10 years ago, a woman was described with breast cancer diagnosed at age 35 years [70]. The family from her mother as well as the family from her father were very suspicious for LS. Mutation analysis demonstrated two missense mutations in the *MLH1*-gene. Since then, more than 25 cases have been described with bi-allelic mutations for all four MMR-genes [71]. The main feature is multiple cafe-au-lait spots which is present in almost all cases. The other hallmark is the development of cancers at an unusually young age (<20 years) including CNS tumours, haematological malignancies and LS-associated tumours (Table 15.5). It is clinically important that the presence of bi-allelic mutations is considered if a member from an LS family develops one of the above mentioned tumours at a unusually young age. In such cases, special attention should be paid to the presence of LS-associated tumours in the family of the other parent.

Table 15.5 Features associated with bi-allelic MMR-gene mutations

Cafe-au-lait spots
Early onset central nervous system tumours
Haematological malignancies
Early onset gastrointestinal neoplasies
Early onset LS-associated tumours
Multiple primary tumours

15.3 Clinical Criteria for the Lynch Syndrome

15.3.1 Amsterdam Criteria I and II

Until 15 years ago, the diagnosis of LS was hampered by the absence of pathognomonic features in contrast with, for example, familial adenomatous polyposis in which the presence of hundreds of colonic adenomas confirms the diagnosis. The diagnosis of LS could only be suspected on the basis of the personal and family history. At that time, the description of the syndrome in the literature varied widely which interfered with comparison of the results of studies. That was the reason for the International Collaborative Group on HNPCC (now called International Society of Gastrointestinal Hereditary Tumours (Insight)) to propose a set of criteria for HNPCC in 1990 (Table 15.6). The aims of the criteria were to allow international collaborative studies and to promote the use of a uniform terminology [6]. In 1999, the International Collaborative Group proposed a second set of criteria [72] that included the extracolonic cancers associated with HNPCC (Table 15.6).

15.3.2 Bethesda and Other Criteria

Over the years, many sets of criteria have been developed for the identification of LS. The most important clinical criteria at present are the so-called Bethesda criteria that were proposed in 1996 [73]. These criteria describe practically all clinical conditions

Table 15.6 Amsterdam criteria II and revised Bethesda criteria

Amsterdam criteria II
There should be at least three relatives with colorectal cancer or with a Lynch syndrome-associated cancer: cancer of the endometrium, small bowel, ureter or renal pelvis
One relative should be a first degree relative of the other two
At least two successive generations should be affected
At least one tumour should be diagnosed before age 50
Familial adenomatous polyposis should be excluded in the colorectal cancer case if any
Tumours should be verified by histopathological examination
Revised Bethesda criteria
Colorectal cancer diagnosed in a patient <50 years of age
Presence of synchronous, metachronous colorectal or other Lynch syndrome-related tumours[a], regardless of age
Colorectal cancer with MSI-H histology diagnosed in a patient <60 years of age
Patient with colorectal cancer and a first-degree relative with a Lynch syndrome-related tumour, with one of the cancers diagnosed under age 50 years
Patient with colorectal cancer with two or more first-degree or second-degree relatives with a Lynch syndrome-related tumour, regardless of age

[a]Lynch syndrome-related tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter, renal pelvis, biliary tract and brain tumours, sebaceous gland adenomas and keratoacanthomas and carcinoma of the small bowel

in which there is a suspicion of LS. If a patient meets one of these criteria, there is an indication for additional molecular genetic studies either by MSI-analysis or immunohistochemical analysis of the mismatch repair proteins (see below). Several studies have shown that these criteria are very useful for the selection of families for mutation analysis [74]. Based on the outcome of these studies and discussions at a National Cancer Institute (NCI) workshop on MSI, held in 2002 in Bethesda, Maryland, USA, the criteria have been updated [11] (Table 15.5).

15.3.3 Tests Performances of Amsterdam and Bethesda Criteria

Previous studies have shown that the yield of mutation analysis (positive predictive value) in families that meet the Amsterdam criteria is approximately 50% and the yield in families that meet the Bethesda criteria between 10 and 20% [75]. A recent analysis showed that the sensitivity of the Amsterdam criteria for the detection of mutations was 40% and that of the (revised) Bethesda guidelines about 90%. This means that if the revised Bethesda guidelines are used, about 10% of the mutation carriers would be missed, mostly patients with CRC diagnosed between ages 50 and 60. According to the revised Bethesda guidelines, in patients with CRC diagnosed in their 50s, special attention should be given to the presence of pathological features that suggest LS. However, the reports that were analysed did not mention the presence of pathology features in the patients with CRC below age 60 years.

These features include tumour-infiltrating lymphocytes, mucinous histology, poor differentiation and Crohn's-like reaction. Recently, a large population-based study comprising 1,098 CRC evaluated the predictive value of these features for the presence of MSI-H tumours [76]. The study showed that all the pathology features listed above were independent predictive factors for the presence of MSI in the tumours with odds ratios ranging from 1.9 to 9.1.

15.4 Molecular Genetic Studies

15.4.1 Mutation Analysis

Due to the heterogeneity of the mutation spectrum in mismatch repair genes, screening for mutations is time-consuming and expensive. Both point mutations and large genomic deletions in the mismatch repair genes have been reported. DNA analysis should therefore include techniques that identify both types of defects. To evaluate the clinical risk factors that best predict the presence of *MLH1* and *MSH2*-mutations, Dutch investigators analysed these genes by density-gradient gel electrophoresis in a large series of kindreds (187) featuring familial clustering of

colorectal cancer (not selected on the basis of MSI or IHC) [75]. Pathogenic mutations were identified in 26% of the families. Multivariate analysis showed that the age of diagnosis of CRC, fulfillment of the Amsterdam criteria I and the presence of endometrial cancer in the kindred were independent predictors of germline mutations of *MSH2* and *MLH1*.

In most European countries, the cost of mutation analysis of all relevant genes, excluding the cost of genetic counselling, is between 1,500 and 2,000 Euro. Fortunately, in contrast to other hereditary cancers, in familial colorectal cancer, cheaper tests, that is, MSI and immunohistochemical analysis of the mismatch repair proteins, are available that can be used to identify families which have a high predicted probability of carrying a mutation. Recently, computer models also have been reported that can be used to predict the probability of finding a mutation.

15.4.2 Microsatellite Instability (MSI) Analysis of Colorectal Tumours

Microsatellite instability (MSI), first reported in 1993, is caused by a failure of the DNA mismatch repair system [77]. Microsatellites are repetitive DNA sequences found throughout the genome. Loss of mismatch repair function may result in mutations in repetitive coding and noncoding regions of genes including those genes involved in tumour initiation and progression. According to international guidelines for the evaluation of MSI, a panel of five microsatellite markers should be used. If two of the five markers show instability, the tumour is referred to as MSI-high (MSI-H). If one of the markers shows instability the tumour is considered MSI-low (MSI-L). A tumour without any instable marker is designated MS-stable (MSS). Because over 90% of colorectal cancers from patients with LS exhibit a high level of MSI, MSI may aid in the diagnosis of this syndrome. However, MSI is not specific to LS, as it also occurs in 15% of apparently sporadic colorectal and other tumours. In these sporadic cases, MSI is caused by hypermethylation of the *MLH1* gene. In 40% of the colorectal cancers with MSI due to hypermethylation, a mutation can be found in *BRAF*-gene whereas this mutation is not found in CRC associated with LS. Thus *BRAF*-analysis can be helpful to distinguish between a somatic event/hypermethylation and a possible germline mutation in the *MLH1* gene.

15.4.3 The Role of Immunohistochemical Analysis of the Mismatch Repair Proteins

Another rapid and cheap technique to identify mismatch repair deficiency is that of immunohistochemical analysis of the mismatch repair proteins in tumours. Using specific antibodies, the presence or absence of the MLH1, MSH2, MSH6 and

PMS2 proteins can be detected [54, 78]. Immunohistochemical staining can be performed on formalin-fixed, paraffin-embedded tissue sections. When analysing MLH1, MSH2, MSH6 and PMS2 expression, tissue stroma and normal epithelium are used as internal controls. Only if there is no nuclear staining of the tumour for one of the antibodies and at the same time normal staining of the internal control tissue, can it be concluded that there is loss of expression of the involved mismatch repair protein.

Since these mismatch repair proteins form heterodimeric complexes, distinct immunohistochemical patterns can be found in tumours of carriers of various mutations. The characteristic pattern found in colorectal tumours from carriers of an *MLH1*-mutation consists of an absence of staining for MLH1 and PMS2 and normal staining for MSH2 and MSH6. This pattern is explained by the fact that the MLH1 protein forms a heterodimer with the PMS2 protein. In the absence of MLH1 protein, the heterodimer will not be formed and the PMS2 protein will degrade resulting in the absence of staining of both proteins. Because MSH2 protein forms a heterodimer with MSH6, the specific immunohistochemical pattern observed in tumours of carriers of an *MSH2*-mutation comprises the absence of staining of MSH2 and MSH6 and normal staining of MLH1 and PMS2. In tumours from carriers of an *MSH6*-mutation, only absence of staining of the MSH6 protein is observed whereas in tumours from carriers of a *PMS2*-mutation, absence of the PMS2 protein is found.

15.4.4 Sensitivity of MSI and IHC for Identification of Mutations

Many studies have been published on the results of MSI or IHC analysis for the identification of MMR-gene mutations. However, most studies have been retrospective and the methods used have varied widely. The number of markers for MSI-analysis ranged from one to more than 10. For IHC-analysis, most studies used two antibodies (MLH1, MSH2) against the MMR proteins; other studies used three or four antibodies (MLH1, MSH2, MSH6, PMS2). A recent analysis of studies in which both MSI-analysis and IHC-analysis have been used prospectively showed that the sensitivity of MSI-analysis was slightly better than that of IHC-analysis [74, 79–86]. One of the largest studies [82] evaluated the outcome of these tests prospectively in families that meet the Amsterdam, Bethesda or slightly modified criteria. In this study, MSI-analysis (using the Bethesda set of five markers) and IHC-analysis (two antibodies) was performed in 1,119 index patients. Altogether 230 pathogenic MMR-gene mutations were identified. The sensitivity of MSI-analysis was 100% and that of IHC-analysis 94%. A Dutch study showed that by adding antibodies against PMS2, the sensitivity for the detection of *MLH1* mutations increased [54]. It is expected that the sensitivity of IHC will increase by using all four antibodies.

15.4.5 Diagnostic Approach in Patients Suspected of Lynch Syndrome

A detailed family history in all patients with cancer is the simplest and most cost-effective way to identify hereditary colorectal cancer. As cancer is a common disease, the occurrence of CRC in several members of one family might be due to clustering by chance. Characteristics of hereditary forms of CRC that might be helpful in the differential diagnosis from non-hereditary cases are an unusually early age of onset, the occurrence of multiple cancers and the combined occurrence of CRC with endometrial cancer or another LS-associated cancer (in an individual or family).

In patients who meet the Bethesda criteria (Table 15.6), the tumour should be analysed by MSI or immunohistochemical analysis of the mismatch repair proteins. The advantage of IHC is that it may direct mutation analysis because the pattern of staining is suggestive for the underlying gene defect. This is the reason for most authors recommending the use of IHC as the first step in families with a high probability of carrying a mutation e.g., families that meet the Amsterdam criteria or families with a high predicted probability based on calculations using computer models [75, 82, 86, 87] (Fig. 15.1). Because of the incomplete sensitivity of IHC-analysis, MSI-analysis is recommended for cases with a high prior probability of LS but with apparently normal expression of the MMR proteins. In families with a moderately increased probability of carrying a mutation, depending on the experience of the centre either MSI- or IHC-analysis might be used as the first step to exclude the presence of MMR deficiency. If IHC-analysis reveals loss of MLH1 expression, DNA-analysis of *BRAF* in the tumour can be performed to distinguish between a somatic event/hypermethylation and a possible germline mutation in the *MLH1* gene. If the specific BRAF V600E mutation is found in tumour DNA, mutation analysis of the *MLH1* gene is not indicated.

Preferably, colon tumour tissue is used for MSI/IHC-analysis. However, if colon tumour tissue is not available other tumours, for example, endometrial cancer or adenomatous polyp may be analysed. Unfortunately, the few studies that are available have shown that sensitivity of MSI/IHC for MMR mutations in endometrial tumour tissues is lower than that of the same analysis of colon cancers [56, 61].

In view of the high costs of testing all CRCs for MSI or loss of MMR-protein, most authors feel that the revised Bethesda guidelines are appropriate tools to help in selecting patients for genetic testing. However, because of the accumulating evidence that MSI is a predictive factor for response to 5FU-based chemotherapy, it is expected that these tests will be performed in an increasing number (if not all) patients with CRC in the near future.

Because interpretation of the pedigree information, the pathology of the tumour and the outcome of MSI and IHC testing can be complex, it is recommended that these data be discussed together by a multidisciplinary team.

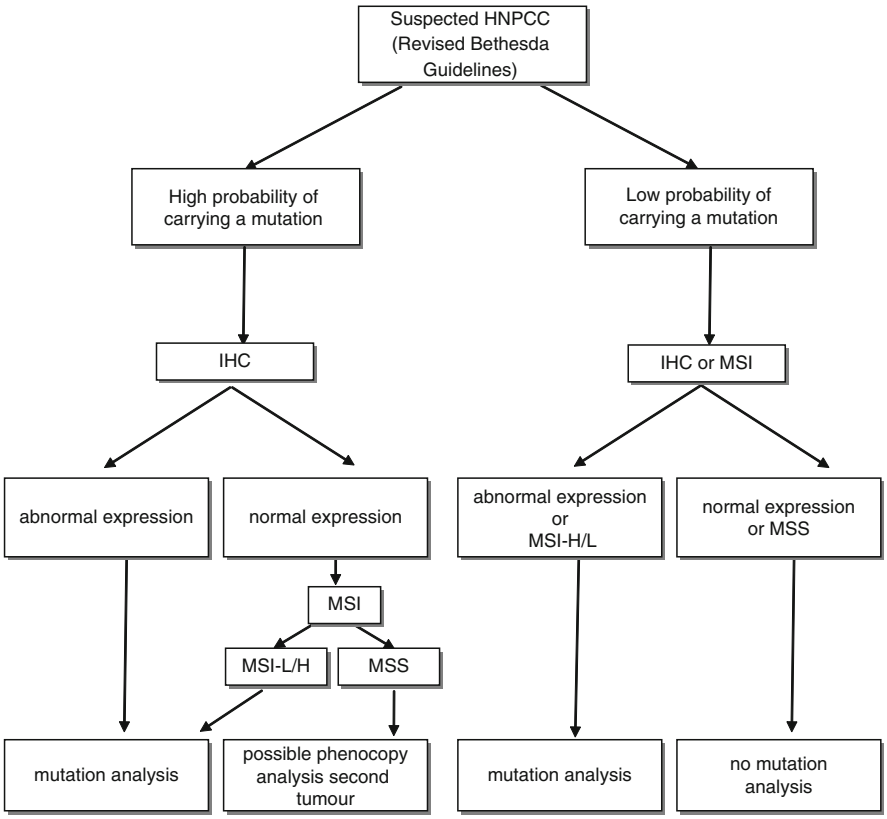

Fig. 15.1 Approach for families with clustering of non-polyposis colorectal cancer or earlyonset colorectal cancer

15.5 Presymptomatic Diagnosis

Detailed information provided by an experienced clinical geneticist and good psychosocial guidance are prerequisites for presymptomatic diagnosis based on DNA-testing. Relatives opting for genetic testing receive one or more individual pre-test counselling sessions. The issues discussed during the first session include the reasons for testing, the clinical features of the hereditary cancer syndrome, the mode of inheritance, the consequences of the test results, the options for treatment in the event of a positive result and the DNA testing procedure. Psychological support is offered to all subjects throughout the testing procedure. Disclosure of the test results follows within 6–12 weeks after blood sampling.

Recent studies showed that the uptake of genetic testing in families with LS varied from 43% in the US, 57% in the Netherlands to 75% in Finland [88–90].

Reasons for this variation might be differences in the study setting. Other reasons might be fundamental differences between the healthcare and social security systems in the United States and Europe. In Europe, where thus far private health insurance has played a minor role, a predictive test for a treatable disease might be more readily accepted.

15.6 Surveillance

15.6.1 Colorectum

Studies have shown that the adenoma–carcinoma sequence may also be applied in development of CRC in LS families. Since the 1980s, colonoscopic surveillance has been recommended for these families. A recent analysis of the literature showed that nine studies have evaluated the effectiveness of colonoscopic surveillance [5, 91–98]. All the studies showed that surveillance led to detection of CRC at an earlier stage compared to the stage in historical controls. The only prospective controlled trial showed that surveillance led to a 63% reduction of CRC [95]. Two studies assessed the effect of surveillance on CRC-associated mortality. A Finnish study showed that colonoscopic surveillance significantly decreased the mortality associated with CRC [68, 95]. A study from the Netherlands evaluated the relative mortality in a large series of families over a period of 45 years. In the Netherlands, a national registry of Lynch syndrome families was established in 1985 to promote the identification of such families and to encourage participation in surveillance programs [5]. Mortality in these families has decreased significantly after the establishment of the registry.

The protocols that have been used in studies of surveillance have varied with respect to the surveillance intervals. Some studies advised a 3-yearly colonoscopy and others colonoscopy every year. A search of the literature did not reveal any studies that compared different surveillance intervals. The Finnish trial showed that 3-yearly colonoscopy significantly reduced colorectal cancer incidence and colorectal cancer-related mortality [95]. Therefore, the only evidence available suggests that a 3-yearly interval may be adequate. However, several observational studies suggest that (interval) cancers can occur within a 3-year interval after a normal colonoscopy. In a Finnish study on 56 families, the stage distribution of colorectal cancer was significantly more favourable in patients ($n = 35$) with cancer detected by surveillance than in patients ($n = 115$) with symptomatic presentation of colorectal cancer [98]. However, a total of 21 cancers were diagnosed after a previous "clean" colonoscopy and half of them were diagnosed within (or at) an interval of three years. These included two Dukes C cancers diagnosed 15 and 20 months after the previous examination. A recent Dutch study on 114 HNPCC families revealed that circa 10% of (mismatch repair) mutation carriers developed colorectal cancer under screening after a follow-up of about 10 years (i.e., a similar percentage

as reported in the Finnish series) [97]. Advanced cancers (Dukes C) were only observed at intervals of longer than two years, whereas all Dukes A and B tumours were detected within an interval of less than 2 years. These observations suggest that the adenoma–carcinoma sequence is accelerated in LS [55, 99]. Therefore, the most appropriate surveillance interval probably lies between 1 and 2 years. In highly selected cases, for example, mutation carriers who have recurrent adenomas, a prophylactic subtotal colectomy may be discussed as option.

Recent studies demonstrated that chromoendoscopic colonoscopy and colonoscopy with narrow band imaging markedly improved the detection of adenomas. The application of these new techniques may prevent the development of CRC in these high-risk patients [100, 101].

In a small proportion of families meeting the Amsterdam criteria, the results of the MSI and immunohistochemical analysis of the colorectal tumour (s) are negative [102]. Clustering of CRC by chance or another genetic defect than a mismatch repair defect may be responsible for the disease in such families. Despite fulfilling the Amsterdam criteria, these families do not have LS. These families are characterized by a lower risk of CRC (RR 2–3), a late age of onset (>50 years) and the absence of endometrial cancer and multiple tumours. In such families, endometrial cancer surveillance is not indicated and a less intensive colonoscopic surveillance programme (e.g., colonoscopy: 1×/3–5 years) might be appropriate.

15.6.2 Surveillance of the Endometrium/Ovary

Previous studies have shown that carriers of an MMR mutation have a high risk of developing endometrial cancer [26]. Although it is known that the majority of (sporadic) endometrial cancers are detected at an early stage because they develop symptoms, about 10–15% of patients with such tumours will ultimately die from metastatic disease. In view of this significant mortality and the high risk of developing endometrial cancer in LS families, most authors advise surveillance of the endometrium.

British and Dutch investigators evaluated the outcome of surveillance of 269 women from families suspected of having LS [103, 104]. The surveillance programme consisted of ultrasound every 1–2 years. It did not lead to the detection of pre-malignant lesions or endometrial cancer. However, two women presented with symptoms at 6 and 24 months after a normal ultrasound and were diagnosed with endometrial cancer. Both tumours were in an early stage (FIGO I). In another study from the Netherlands, 41 women from LS families underwent surveillance by transvaginal ultrasound followed by aspiration biopsy in suspected cases. After a mean follow-up of 5 years, premalignant lesions, that is, complex atypia, were detected in three patients. There was one interval cancer diagnosed 8 months after a normal ultrasound. This tumour was at an early stage. A recent study of 175 subjects from Finland reported the results of surveillance by transvaginal ultrasound (TVU) and aspiration biopsy [105]. Complex atypia was found in five patients, endometrial

cancer was found in 11 and there were two interval cancers. Six of the eleven screen-detected cancers were only identified by aspiration biopsy and not by TVU.

American investigators reported on a retrospective cohort of 315 women, all mutation carriers, 61 of whom had prophylactic surgery and were then followed up for approximately 10 years. No endometrial cancer or ovarian cancer developed in those women who had prophylactic surgery whereas 33% of women who did not have surgery developed endometrial cancer and 5.5% developed ovarian cancer [106].

In conclusion, two of the three available studies suggested that surveillance may lead to detection of pre-malignant lesions and one study also to the detection of endometrial cancer at an early stage. More prospective studies are needed to establish the most appropriate screening protocol. Because of the higher risk of developing endometrial cancer in carriers of an *MSH6*-mutation, hysterectomy may be suggested to these women after menopause. This surgery may also be considered for carriers of mutations in the other MMR genes and for women who require surgery for CRC. In view of the risk of ovarian cancer and the failure of early detection of such tumours by TVU and CA-125 estimation, bilateral salpingo-oophorectomy might be considered in mutation carriers after completion of the family.

15.6.3 Surveillance for Other Related Cancers

Other cancers associated with LS include cancer of the stomach, ureter, renal pelvis, small bowel, bile ducts and tumours of the brain. The lifetime risk of developing one of these cancers is relatively low (less than 10%) and may vary with the underlying MMR defect. The risk of developing gastric cancer may be higher in some countries. The International Society of Gastrointestinal Hereditary Tumours (InSiGHT) recommends surveillance for cancer of the stomach, if the cancer clusters in the family (more than one case) [107]. However, others recommend surveillance in LS families for gastric cancer in countries with a high incidence of such tumours.

In the decision-making process regarding which surveillance protocol should be recommended, a reasonable approach might be first to discuss all the various cancer risks with the patient, then discuss which screening protocols are established as effective based on published evidence, for example, colon and possibly endometrium screening (see above). Finally, the physician and patient should weigh up the possible benefits versus costs and risks for screening for other cancers. In addition, it should be recommended to all at-risk family members that they should contact a physician early if they are worried about specific signs or symptoms.

The guidelines for surveillance of LS families recently reported are summarised in Table 15.7. These protocols are indicated not only in families with an identified MMR defect but also in families with clustering of CRC and other related cancers with evidence of mismatch repair deficiency, for example, by the presence of MSI or loss of expression in tumours (with the exception of families of patients with such features caused by hypermethylation of *MLH1*).

Table 15.7 Recent published surveillance protocols in Lynch syndrome

		Lindor et al. (JAMA 2006)	Hendriks et al. (CA Cancer J Clin 2006)	Vasen et al. (J Med Genet 2007)
Colorectum	Examination	Colonoscopy	Colonoscopy	Colonoscopy
	Lower age limits	20–25 years (MSH6: from age 30 years) or 10 years younger than the youngest age at diagnosis in the family whichever comes first	20–25 years (*MSH6*: from age 30 years)	20–25 years
	Interval	1–2 years	1–2 years	1–2 years
Endometrium	Examination	Transvaginal ultrasound, endometrial biopsy	Ultrasound, CA-125 analysis	Gynaecological examination, transvaginal ultrasound, endometrial biopsy
	Lower age limits	30–35 years	30–35 years	30–35 years
	Interval	1 years	1–2 years	1–2 years
Stomach	Examination	–	Gastroduodenoscopy[1]	Gastroduodenoscopy[2]
	Lower age limits	–	30–35 years	30–35 years
	Interval	–	1–2 years	1–2 years
Upper urinary tract	Examination	Urinalysis with cytology	Urine cytology[1]	Abdominal ultrasound, cytology urine[2]
	Lower age limits	25–35 years	30–35 years	30–35 years
	Interval	1–2 years	1–2 years	1–2 years
Prophylactic surgery		Discuss hysterectomy or oophorectomy as option after childbearing completed	Consider hysterectomy in *MSH6* above age 50 years	Discuss prophylactic hysterectomy and salpingo-oophorectomy as option
Comments		History and examination with detailed review of systems, from age 21 years, annually	If there are two or more gastric cancers or upper urinary tract cancers in the family[1]	If gastric or urinary tract cancer runs in the family[2]

15.7 Treatment

15.7.1 Surgical Management of Colorectal Cancer

Several studies have shown that LS patients have an increased risk of developing multiple (synchronous and metachronous) CRCs. Thus, before resection of a colon tumour, it is important to visualize the complete colon because of the risk of a synchronous tumour.

A Dutch study reported that the risk of developing a second colon tumour after treatment of a primary colorectal cancer in LS was 16% after 10 years of follow-up [97]. In view of this substantial risk, the question arises whether a subtotal colectomy instead of a segmental resection might be the preferred treatment in patients from LS families with a primary tumour. In another Dutch study, a decision analysis was performed to compare the life expectancy for patients undergoing subtotal colectomy or partial resection for a primary screen-detected colorectal cancer [55]. The results indicated that subtotal colectomy performed at a young age (≤47 years) would lead to an increased life expectancy of up to 2.3 years. Unfortunately, the authors were not able to use quality of life (QOL) adjusted life expectance because studies on QOL that specifically consider LS patients were not available in the literature. Although for sporadic CRC, QOL after segmental resection has been reported to be better than after subtotal colectomy, in LS families, QOL after segmental resection may be decreased by the need for colonoscopy (versus sigmoidoscopy after subtotal colectomy) and the fear of a second tumour.

Based on these findings plus the substantial risk of developing a second tumour, subtotal colectomy with ileorectal anastomosis can be considered if colon cancer is detected in a young patient participating in a surveillance programme. A prospective study that also addresses QOL should evaluate which surgical option is the most appropriate in LS. Until the outcome of such studies is available, most authors recommend discussing the pros and cons of both options with a patient from a LS family who develops CRC.

15.7.2 Chemotherapy

Currently, at least three chemotherapeutic agents have been proven to be effective in the treatment of colorectal cancer, that is, 5FU with or without leucovorin, oxaliplatin and irinotecan (CPT11). Unfortunately, the effectiveness of these agents in patients with MSI-H CRC or LS CRC tumours is unknown. In vitro-studies suggested that MMR-deficient colon cancer cells might not respond to 5FU-based chemotherapy [108]. On the other hand, CRC cell lines defective of MMR exhibit increased sensitivity to CPT11 (irinotecan) [109].

Therefore, the question is whether chemotherapy is effective in patients with MSI-H tumours. The effect of chemotherapy in patients with MSI-H or HNPCC

tumours has been reported in only a few studies [110–114]. Most studies showed that there was no benefit of 5FU treatment in such patients. One small study on Stage IV CRC-patients reported complete or partial responses to treatment with irinotecan in four out of seven patients with MSI-H tumours compared to seven out of 65 patients with MSI-L/MSS tumours [114].

Because most studies are retrospective, all authors urge caution in incorporating these findings in clinical decision making until they are confirmed by prospective studies. Because it may be unethical to withhold chemotherapy in a clinical trial for potentially curable advanced-stage colon cancer, the best format of such studies is to compare effective drugs such as CPT11 or oxalaplatin with 5FU.

15.8 Chemoprevention

There is much evidence that LS may be susceptible to environmental manipulation, as demonstrated by the decrease in the incidence of gastric cancer and perhaps also by the apparent differences in penetrance between men and women.

A recent study evaluated associations between dietary factors – cigarette smoking and LS-associated colorectal tumours in a Dutch case-control study. Fruit consumption was inversely associated with developing LS-associated tumours. A borderline significant inverse association was observed for dietary fibre intake. Cigarette smoking was found to increase the risk of LS-associated tumours. The observed associations support the hypothesis that LS-associated outcomes might be modified by environmental factors.

There is interest at the moment regarding the role of aspirin in bowel cancer prevention. Several large studies have demonstrated that aspirin reduces the risk of bowel cancer in the general population. There is separate evidence that suggests that resistant starch (an isomer of starch) may also play a role in reducing bowel cancer risk. However, a recent randomized, placebo-controlled trial showed that aspirin and/or resistant starch has no effect on incidence of adenoma or carcinoma among carriers of Lynch syndrome [115].

15.9 Conclusions

The identification of individuals predisposed to colorectal cancer is important, as it makes it possible to prevent significant morbidity and early death associated with advanced cancer. Although a variety of molecular tests are available to assist in or confirm the diagnosis, the mainstay of diagnosis remains an accurate family history. Until recently, the Amsterdam criteria were the most important tool for the identification of LS. However, since we know that the LS is caused by a mismatch repair defect and that the hallmark of the syndrome is MSI, more attention should be given to the Bethesda criteria that describe all

clinical conditions in which a search for MSI is indicated. All specialists that are involved in the treatment of cancer patients should know these criteria in order to identify all families suspected of the LS. Using MSI-analysis or immunohistochemical analysis of the mismatch repair-proteins, patients with a high probability of carrying a mutation can be identified and these patients should be referred to family cancer clinics for genetic testing. Genetic testing should only be performed after providing full information about the pros and cons of testing and under good psychosocial guidance.

At present, MSI and immunohistochemical analysis are advised only in families that comply with specific criteria. Because, there is increasing evidence that MSI is an important prognostic factor and may predict the response to chemotherapy, in the near future these tests might be performed on a much larger scale if not in all colorectal cancer cases.

Treatment is chiefly by means of surveillance, and regimens will vary with local resources. The surveillance programs are life long once started. To promote maximal compliance with the recommended surveillance protocols, careful education and counselling about all details of the disease are essential. Experience has shown that long-term surveillance of high-risk families cannot be adequately guaranteed by individual specialists, and this can lead to considerable morbidity and mortality. In several countries, these problems have inspired specialists to establish national and regional registries that monitor the continuity of the surveillance programs by periodic assessment of the screening results. The registries also ensure that the same protocol is offered to the various branches of the families that are followed-up by different specialists. Hereditary cancer registries also have a role in the assessment of the results of long-term surveillance. This is important, as the value of most suggested protocols is as yet unknown.

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Chapter 16
Surgical Management in Lynch Syndrome

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Abstract Surgery in the Lynch Syndrome can be curative, palliative, and occasionally prophylactic or preventive. An abdominal colectomy and ileorectal anastomosis is the usual procedure recommended in a patient with newly diagnosed colon cancer and Lynch Syndrome. This recommendation is based on the increased risk of metachronous colorectal cancer in the syndrome. There is a paucity of studies regarding the benefits of more extended procedures compared to limited resections in the Lynch Syndrome. In this chapter, the surgical management of Lynch syndrome patients will be addressed including the rationale for limited and extended resections.

Keywords Lynch Syndrome • HNPCC • Surgery • Segmental resection • Abdominal colectomy • Ileorectal anastomosis • Prohpylactic Surgery

16.1 Introduction

Hereditary Non-Polyposis Colorectal Cancer (HNPCC) or Lynch Syndrome is the most common hereditary colorectal cancer syndrome. Colorectal cancer (CRC) is the most common malignancy associated with the Lynch Syndrome. The lifetime risk of CRC in individuals with an identified mismatch repair mutations has been reported to be between 28–75% in males and 24–52% in females [1]. Endometrial cancer is the second most common cancer in HNPCC. The lifetime risk of endometrial cancer has been reported to be 27–71% which in some series is higher than the colorectal cancer risk in females [1]. As such, the practicing clinician will need to identify and manage these patients. Surgery in the Lynch Syndrome can be curative, palliative, and in some cases, prophylactic or preventive. Recommendations regarding the best surgical approach for HNPCC patients with colorectal cancer are based on limited scientific

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knowledge as prospective studies and retrospective studies are lacking. In this chapter, we will address the surgical management of patients with the Lynch Syndrome.

16.2 Colon and Rectum

16.2.1 Surgery for a Newly Diagnosed Tumor

The majority of newly diagnosed patients with colorectal cancer and HNPCC present with neoplasms proximal to the splenic flexure. Right-sided cancers are the index colorectal cancer in up to 70% of Lynch Syndrome patients with colorectal cancer [2]. Synchronous CRC in HNPCC have been reported to occur between 6 and 18% while the risk of metachronous CRC has been estimated at 40 and 72% at 10 and 40 years following resection of the primary CRC tumor, respectively [2–4]. In the Netherlands, the risk of metachronous CRC after segmental resection has been reported to be 16% at 10 years [5].

As with any patient with colorectal cancer, patients with HNPCC and colorectal cancer should have a complete pre-treatment evaluation to clinically stage the tumor. In our practice, in addition to the history and physical examination, all patients will undergo colonoscopy, chest radiography, and computed tomography of the abdomen and pelvis. If the tumor is in the rectum, an endorectal ultrasound or an MRI to evaluate for depth of penetration into the bowel wall and lymph node involvement is performed.

16.2.2 Colon

Because of the increased incidence of synchronous and metachronous CRC, an abdominal colectomy with an ileorectal anastomosis (IRA) (Fig. 16.1) as opposed to a segmental has been recommended as the procedure of choice in HNPCC patients with newly diagnosed colon cancer. However, there are no reports of prospective and/or retrospective studies demonstrating a survival benefit in patients undergoing IRA compared to those undergoing segmental resection. In a retrospective study it was reported that patients undergoing segmental resection had a higher incidence of metachronous colorectal cancer as well as a higher incidence of re-operation compared to those undergoing more extended procedures [5]. However in this study, there was no evidence of improved survival in patients undergoing more extended procedures than those undergoing limited resection. A mathematical model has been published comparing the estimated life expectancy in HNPCC patients with CRC undergoing segmental versus more extended resections [6]. Not unexpected, life expectancy was better in younger patients with early CRC undergoing more extended procedures when compared to older patients and to patients with lymph node positive disease irrespective of their age where the benefit was modest [6].

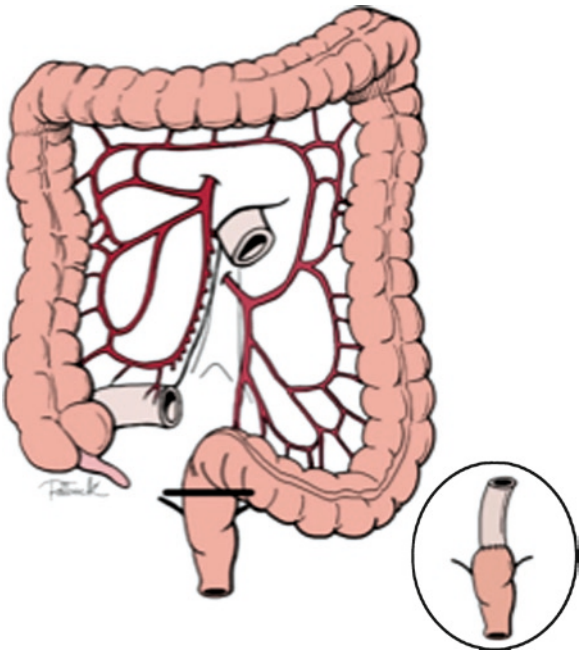

Fig. 16.1 Total colectomy and intraperitoneal ileorectal anastomosis

In this model, quality of life was not taken into account. Quality of life was taken into account in another mathematical model reported by Maeda et al. [7]. In this model, when quality-adjusted life years were taken into account, both segmental resection and abdominal colectomy were approximately equivalent. The authors concluded that patient factors and preferences should be the main factors considered in the surgical decision making of a patient with colon cancer and Lynch syndrome.

The disadvantage of an abdominal colectomy with an ileorectal anastomosis compared to a segmental resection is mainly in the bowel frequency. Patients undergoing IRA will have more bowel movements compared to a patient undergoing a segmental colectomy. Over time, patients either adapt or get used to the frequency of bowel movements. Even though IRA is a more extended procedure than segmental resection, it still has a low morbidity and mortality [8]. It must be understood that this procedure does not prevent rectal cancer, which has been estimated to occur between 3 and 12% of the cases at 12 years [9, 10]. Therefore, IRA patients need an annual rectal endoscopy to diagnose small adenomas or low-stage adenocarcinomas [10].

16.2.3 Rectum

When an HNPCC patient presents with a primary rectal cancer, the issue becomes whether the sphincter muscles can be saved and a restorative procedure performed. If the sphincter can be saved, then the rectal tumor should be addressed as any other rectal cancer.

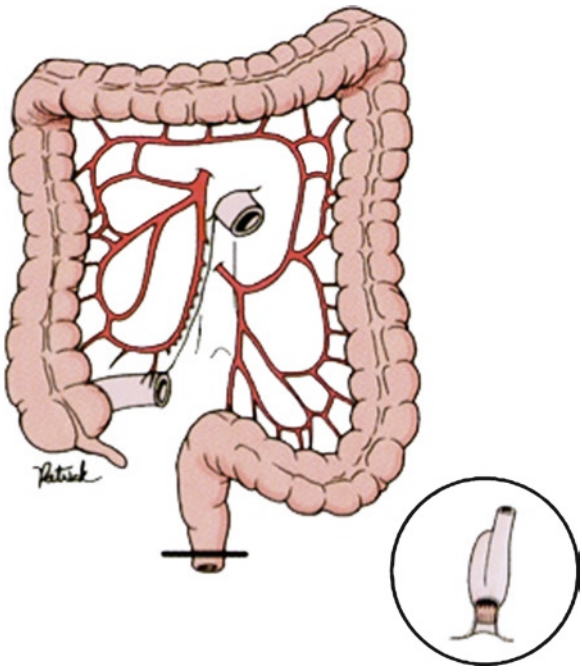

Fig. 16.2 Total proctocolectomy with ileoanal anastomosis and ileal pouch

If in the pre-treatment evaluation it is estimated that the patient would need neoadjuvant therapy, then the surgical procedure is performed after such therapy.

If a restorative procedure can be performed, then in general a restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) is preferred (Fig. 16.2). However, as in the colon, a segmental resection such as a proctectomy and a coloanal anastomosis, a low anterior resection, or if the sphincters are involved, an abdominoperineal resection or more extended procedure such as total proctocolectomy with an ileostomy can be performed. The advantage of a restorative proctocolectomy is that this surgical procedure simultaneously addresses the primary lesion and prevents metachronous large bowel tumors. A temporary loop ileostomy is routinely performed and closed 3 months later if there is no leak or stricture at the anastomotic site. Although IPAA may be the most appropriate approach for both treatment and prevention, it is followed by undesirable morbidity, with increased bowel movements, at times inability to differentiate stool from gas, and soiling in more than 30% of the cases [11, 12]. Other complications include intestinal obstruction, which has been observed in 13% of the patients operated on for familial adenomatous polyposis [13]. In addition, the morbidity of reversing the ileostomy has to be taken into account. Alternatively, these patients may opt for a lesser procedure where just the segment of the rectum affected with the cancer is removed and an anastomosis is performed if the sphincter can be spared. These procedures have lesser issues with bowel function when compared to IPAA, but still bowel function is not perfect. It is understood that if a segmental procedure is performed, the patient will need annual endoscopic surveillance (Fig. 16.3).

16.3 Previous Segmental Resection

It is not unusual to encounter an HNPCC patient who has undergone a segmental resection. These patients undergo segmental procedures either because of no previous recognition of the family syndrome or as a consequence of the patient or surgeon preference. The management of these individuals includes either a completion colectomy and IRA, or annual or biennal colonoscopies. However, there is no strong data to support the superiority of any of these approaches over the other (Fig. 16.4).

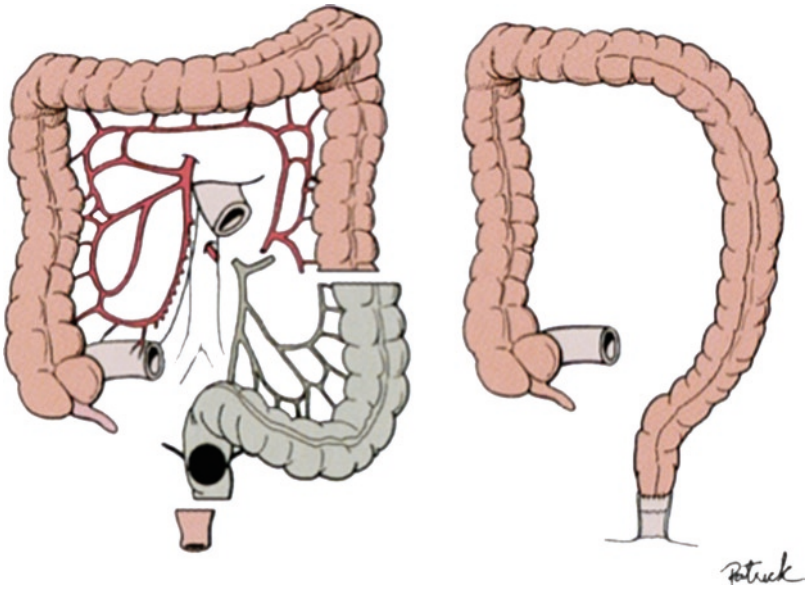

Fig. 16.3 Rectossigmoidectomy with colorectal anastomosis

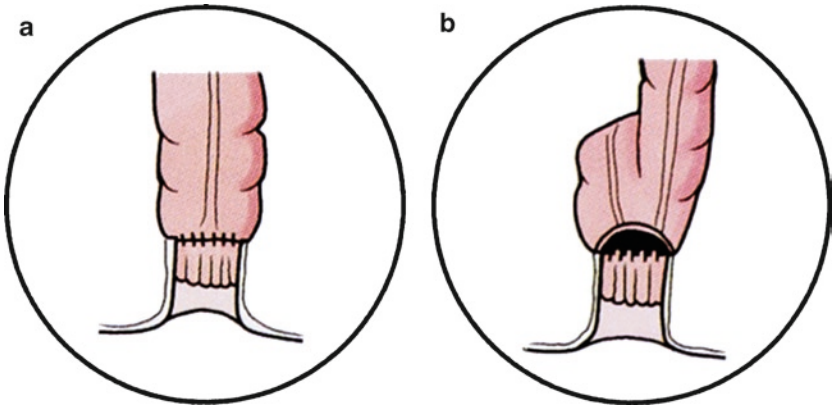

Fig. 16.4 Segmental resection for low rectal cancer with a primary anastomosis. (**a**) straight anastomosis, (**b**) J-colon pouch

16.4 Mutation Carriers with Adenomas

The adenoma to carcinoma sequence is accelerated in patients with Lynch syndrome compared to sporadic adenoma patients [14, 15]. The treatment options for an HNPCC individual with adenomas include endoscopic polypectomy (if technically possible) and continued rigorous surveillance, or surgical resection as described for an HNPCC-affected individual with colorectal cancer. Factors to consider when deciding on surveillance versus surgery include the size and number of the adenomas, the frequency of recurrent or metachronous adenomas, the risk of interval cancer, the morbidity of endoscopic polypectomy, and the risk of prophylactic surgery.

16.5 Gene Mutation Carriers

In familial adenomatous polyposis, prophylactic colectomy has been shown to improve the survival of affected individuals [16]. There is no similar data in HNPCC patients. However, there are mathematical models suggesting a modest benefit in survival compared to surveillance [17, 18]. In one of these models, the predicted survival benefit was 12 and 24 months if colectomy was performed at age 30, whereas in the other model, the survival benefit was calculated to be 19.6 months if colectomy was performed at age 25 [17, 18].

In patients with poor compliance with surveillance or those with a disabling psychological impact from the fear of developing cancer, a prophylactic abdominal colectomy with ileorectal anastomosis should be considered. The patient should understand that the surveillance of the rectal stump must be continued. Laparoscopic colectomy is feasible in these individuals [19].

16.6 Patients with Microsatellite Unstable Colorectal Cancer (MSI-H)

The presence or absence of microsatellite instability (MSI-H) in a colorectal cancer biopsy specimen could possibly guide the choice of surgical procedure in an individual without family history of colorectal cancer. In approximately 30% of patients diagnosed with CRC at age less than 30, a germline mutation will be found in either MLH1 or MSH2 [20, 21]. In this age group, an MSI-H colorectal cancer doubles the chances of finding a germline mutation in either MLH1 or MSH2 [20, 21]. In patients diagnosed with colorectal cancer at age <50 whose tumors have MSI-H, the chance of finding a germline mutation in either MLH1 or MSH2 is approximately 30% [21, 22]. Therefore, an argument can be made to proceed with an abdominal colectomy in very young patients or in patients diagnosed at age <50 years whose tumors are MSI-H. Every situation is different and should be individualized. Older individuals with MSI-H tumors most likely will have hypermethylation of the promoter of MLH1 and thus be considered sporadic.

16.7 Prophylactic Hysterectomy and Salpingoophorectomy

Women from HNPCC families have a 27–71% cumulative risk of developing endometrial cancer by age 70 [23]. In MSH6 mutation carriers, the incidence of endometrial cancer is higher than colorectal cancer [23]. The mean age of diagnosis of endometrial cancer has been reported to be 48 years, 49 years, and 54 years in MLH1, MSH2, and MSH6 mutation carriers, respectively [23]. Prophylactic hysterectomy and bilateral salpingoophorectomy should be discussed with mutation carriers who are undergoing abdominal surgery who are pre-menopausal and have completed their families or in those who are post-menopausal. There is retrospective data to suggest that women who undergo prophylactic surgery decrease their incidence of endometrial and ovarian cancer in HNPCC. In a study of over 300 females with mismatch repair gene mutations, 61 patients who underwent prophylactic surgery did not develop endometrial or ovarian cancers whereas 33% and 5.5% of those who did not undergo prophylactic surgery developed endometrial and ovarian cancer on follow-up, respectively [24]. In patients undergoing risk reducing bilateral salpingoophorectomy, estrogen replacement therapy may be administered as there is no evidence that the incidence of other cancers is affected [25].

There are no prospective studies evaluating the efficacy of surveillance in endometrial cancer in HNPCC patients. Endometrial cancer surveillance has been recommended as early as age 25 years [26]. Others have recommended annual transvaginal ultrasound and endometrial sampling beginning at age 30–35 [27]. Transvaginal ultrasound appears not to be not effective in endometrial cancer surveillance due to the high false positive rate [28]. However, it may be useful in ovarian cancer surveillance [27].

16.8 Conclusions

Although in the last 10–15 years, new clinical and genetic knowledge has oriented surgeons to pursue the best surgical options for HNPCC patients, there is no procedure that suits all patients. Each situation has to be analyzed individually. In the future, new frontiers will be conquered and will help surgeons and clinicians define subgroups of patients that can better benefit from major resections, prophylactic surgery or, instead, colonoscopic or other surveillance modalities.

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Chapter 17
Extracolonic Tumors

Benedito Mauro Rossi and Fabio de Oliveira Ferreira

Abstract Lynch Syndrome (LS), or Hereditary non-polyposis colorectal cancer (HNPCC), is caused by inherited germline mutations in mismatch repair (MMR) genes. It is one of the commonest forms of inherited predisposition to colorectal cancer (CRC), accounting for 2%–5% of all CRC. LS is characterized by early age of onset, with a tendency for multiplicity of tumors, and an increased risk for extracolonic tumors at particular sites. In this chapter, the LS extracolonic tumors characteristics are presented, including tumor spectrum and lifetime risk of cancer. Some specific types of cancer, such as endometrium, urinary tract, small bowel, brain, stomach, hepatobiliary tract, pancreas, skin and breast are discussed in detail.

Keywords Lynch syndrome • Hereditary tumors • Colorectal cancer • Extracolonic cancer • Tumor spectrum • Lifetime risk

The term *Hereditary Nonpolyposis Colorectal Cancer*, or HNPCC, has been less used for naming the classical autosomal dominantly inherited susceptibility to cancer [1]. As this susceptibility applies to tumors from different primary sites other than but including colorectal cancer (CRC), the term *Lynch Syndrome* (LS) is a less restrictive name. *Lynch Syndrome* is characterized by an autosomal dominantly inherited susceptibility to nonpolyposis colorectal carcinoma with early age of onset, predilection to the proximal colon, and multiple primary CRCs; it is also associated to extracolonic cancer, particularly endometrium carcinoma (EC) [2, 3].

Historically, since the first description of the G family, extracolonic tumors had been related to inherited susceptibility to cancer. To establish a profile of the disease, a better definition of the spectrum of related tumors has been a constant

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concern, but some factors make difficult an absolute definition, among them: different repair genes involved, population genetic variations, and gene–environment interactions [4, 5]. It is therefore expected to find heterogeneity among the families regarding the susceptibility to develop extracolonic tumors in different specific sites. The risk of extracolonic cancer in fact varies among families with LS, although the variation does not necessarily result from genetic heterogeneity. The standards of environmental exposure must contribute to the differential gene expression, justifying at least in part this heterogeneity [6].

LS accounts for 1–5% of all CRC cases [7]. In fact, it is believed that 20 to 30% of patients with CRC present some type of genetic susceptibility, but without meeting criteria for known typical syndromes. However, new cancer cases in the patient's family or supplemental information on previously unknown cases can lead to a reclassification that may characterize a typical syndrome. In other situations, despite the lack of clinical criteria for determining an inherited character, molecular inquiry can define the diagnosis of inherited syndrome. For these reasons, even in the absence of typical clinical characterization, criteria must be used to direct the inquiry of an inherited condition. In this context, the correct judgment of the spectrum of extracolonic tumors in LS acquires great importance; however, in the words of Henry Lynch, "the full tumor spectrum remains enigmatic."

17.1 Tumor Spectrum

Besides the importance of clinical diagnosis, the determination of the spectrum of tumors that are part of LS is crucial for establishing screening and follow-up programs. In the 1980s, different primary sites had been described in families with a possible diagnostic of LS: ovary, central nervous system (CNS), hepatobiliary tract, small bowel, urinary tract, breast [8, 9], stomach [10], pancreas [11], and the lymphatic and hematopoietic systems [12].

The spectrum of extracolonic tumors in LS began to be the subject of several publications in which the most common cancers found were those affecting the endometrium, the stomach and the urinary tract [13–19]. Watson et al. [20] evaluated family data from three great datasets of LS, having demonstrated a cumulative risk of 30% for the development of endometrial cancer in LS gene carriers by age 70. Watson and Lynch [6] calculated the frequency of cancer in other specific sites in 1,300 high-risk individuals from 23 families having LS and demonstrated a significant increase of the risk of developing cancer in the stomach (RR:4.1), small bowel (RR:25), kidneys (RR:3.2), ureter (RR:22), and ovary (RR:3.5).

Taking into account the ample heterogeneity of presentation and with the aim of making possible uniformity on the description of LS in collaborative studies, Vasen et al. [21] reported that one of the first successful efforts of the *International Collaborative Group on HNPCC* (*ICG-HNPCC*) at its meeting in Amsterdam in 1990 was the establishment of a set of selection criteria for families with LS. But some investigators feel that the criteria exclude some classic LS families because

they do not take into account extracolonic cancers that are part of the syndrome and many true LS families would be missed. In 1997, in Noordwijk, The Netherlands, and 1998, in Coimbra, Portugal, new selection criteria were proposed that included extracolonic cancers associated with LS: endometrium, stomach, ovary, small bowel, ureter, renal pelvis, brain, and hepatobiliary tract. Among these tumors, endometrium, ureter, renal pelvis, and small bowel cancers present the highest relative risk, and are therefore the most specific for LS.

At the 1998 meeting, agreement was reached that cancer of the endometrium, ureter, renal pelvis, and small bowel should be included, and a set of new clinical criteria was then proposed (Amsterdam Criteria II [ACII]) [21]. In addition, it was proposed to keep the classical criteria, which are still considered useful by a large number of members, referred to as Amsterdam Criteria I [ACI] [22]: (1) at least three relatives must have histologically verified CRC; (2) one must be a first-degree relative of the other two; (3) at least two successive generations must be affected; (4) at least one of the relatives with CRC must have received the diagnosis before age 50; and (5) familial adenomatous polyposis must have been excluded. Because there are families with an MMR mutation present exclusively in patients with endometrial cancer without CRC, the requirement of at least one case of CRC was suppressed (Table 17.1).

Patients with LS may also have sebaceous adenomas, sebaceous carcinomas, and multiple keratoacanthomas, findings consonant with Torre's syndrome variant [23, 24]. The ICG definition of LS includes a familial clustering of colorectal and/or endometrial cancer and as associated cancers stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin (sebaceous tumors) tumors [21, 25].

In LS families reported in Western countries, stomach cancer is uncommon. In Asian countries such as Japan and Korea [26] and in Brazil [27], however, a very high incidence of stomach carcinoma is reported. Unfortunately, there are no studies in these countries showing the relative risk of developing stomach cancer in

Table 17.1 Criteria for LS clinical diagnosis [21]

Amsterdam criteria I [ACI]	Amsterdam criteria II [ACII]	Extracolonic cancers associated with LS
At least three relatives must have histologically verified CRC	Amsterdam I Extracolonic tumors Endometrium Ureter and renal pelvis Small bowel OBS: Because there are families with an MMR mutation with exclusively patients with endometrial cancer without CRC, the requirement of at least 1 case of CRC was omitted	Endometrium Stomach Ovary Small bowel Ureter and renal pelvis Brain Hepatobiliary tract Skin (sebaceous tumors)
One must be a first-degree relative of the other two		
At least two successive generations must be affected		
At least one of the relatives with CRC must have received the diagnosis before the age of 50 years		
Familial adenomatous polyposis must have been excluded		

people having a mutated MMR gene. The high prevalence of this cancer in the whole population in some Asian countries may occasionally result in the chance of familial aggregation of CRC and stomach cancer.

As discussed by Vasen [28], since it is known that LS is caused by an MMR defect and that the hallmark of the syndrome is microsatellite instability (MSI), more attention should be given to the so-called Bethesda guidelines, which describe almost all clinical conditions in which there is suspicion of LS and in which a search for MSI is indicated.

17.2 Lifetime Risk of Cancer

Some studies of people having LS demonstrated a 78% cumulative risk for CRC, 40–60% for endometrial cancer, 19% for gastric cancer, 17.5% for biliary tract cancer, 10% for urinary tract cancer, and 10–12% for ovary cancer. The risk of a metachronous tumor can reach as much as 90% after CRC treatment and 75% after endometrial cancer treatment. The second most common primary tumor is another CRC or another endometrial cancer [5, 7, 29] (Table 17.2).

Vasen et al. [30] also studied age-specific cancer risk in a large series of MMR gene carriers. Significant heterogeneity in phenotypic expression of extracolonic cancer between MLH1 and MSH2 carriers has been found. Gender differences in CRC and extracolonic cancer expression within the MSH2 genotype were also noted. Thirty-four families were studied by mutation analysis, and in 19 of these families, pathogenic mutations were found at hMSH2 or hMLH1. Of 382 relatives, 124 had a mutation in hMLH1 and 86 in hMSH2. The lifetime risk of CRC was the same in both groups of gene carriers (80%). The risk of endometrial cancer was greater in hMSH2 gene carriers compared with hMLH1 gene carriers (61% vs. 42%) but the difference was not statistically significant. A very high relative risk of cancer of the small bowel (relative risk > 100) was observed in carriers of either gene. However, only the carriers of hMSH2 mutations had a significantly increased relative risk of urinary tract cancer – kidney and ureter –

Table 17.2 Age-related cumulative risk (%) for CRC and extracolonic cancer in LS [7]

Age (years)	CRC	Endometrial cancer	Gastric cancer	Bile tract cancer	Urinary tract cancer	Ovarian cancer
20	0.3	0	0	0	0	0
30	6.6	0	0	0	0	0
40	24.3	3.7	1.3	0.3	0.3	1.8
50	46.4	17.1	2.9	1.5	0.8	7.0
60	59.1	35.9	8.8	5.6	2.7	9.0
70	67.7	39.0	14.7	6.8	4.6	9.0
80	78.4	42.6	18.9	17.5	10.2	9.0

(relative risk of 75.3), stomach cancer (relative risk of 19.3), and ovary cancer (relative risk of 8.0).

In the study of Lin et al. [31] aimed at determining the penetrance of CRC and extracolonic tumors in LS mutation carriers, 49 patients were identified as having a MSH2 germline mutation, and 56 patients were identified as having an MLH1 mutation. Lifetime risk of extracolonic cancers in MSH2 and MLH1 carriers was 48% and 11%, respectively. Extracolonic cancer risk in MSH2 female and male carriers was significantly different (69% and 34%, respectively). Mean age of extracolonic cancer diagnosis was significantly higher for MSH2 males than females (55.4 vs. 39.0). No differences in risks for CRC and extracolonic cancer between MLH1 females and males were identified. The risk of extracolonic cancer by age 60 was greater in MSH2 mutation carriers than in MLH1 ones. Gender differences in CRC and extracolonic cancer risk were observed for MSH2 carriers only.

Hendriks et al. [32] examined the cumulative risk of developing cancer in a total of 146 MSH6 mutation carriers. The cumulative risk for CRC was 69% for men, 30% for women, and 71% for EC at 70 years of age. The risk for all LS-related tumors was significantly lower in MSH6 than in MLH1 or MSH2 mutation carriers. In female MSH6 mutation carriers, the risk for CRC was significantly lower and the risk for endometrial cancer significantly higher than in MLH1 and MSH2 mutation carriers. In male carriers, although the risk for CRC was lower in MSH6 mutation carriers, the difference was not statistically significant.

Vasen et al. [33], comparing the risk of developing CRC, EC, and other cancers among families with the various MMR-gene mutations, found that lifetime risk of developing cancer at any site was significantly higher for MSH2 than for MLH1 mutation carriers. The risk of developing colorectal or endometrial cancer was higher in MSH2 than in MLH1 mutation carriers, but the difference was not statistically significant. MSH2 mutation carriers were found to have a significantly higher risk of developing cancer of the urinary tract. The risk of developing ovary, stomach, and brain cancer was also higher in MSH2 than in MLH1 mutation carriers, but the difference was not statistically significant.

In a study of Plaschke et al. [34], the analysis of the involvement and phenotypic manifestations of MSH6 germline mutations in families suspected of LS showed that in about half of the families at least one patient developed CRC or EC in the fourth decade of life.

Currently, cancer risks for individuals with LS are based on data from clinically ascertained families. Hampel et al. [35] studied the penetrance in LS using a comprehensive dataset from a geographically defined region. A combined dataset of 70 LS families ascertained by traditional high-risk criteria and by molecular screening, comprising 88 probands and 373 mutation positive family members, was used. Median age of onset of EC was 62.0 years (CI, 55.9 years to an upper limit too high to calculate) with a lifetime cancer risk of 54% (CI, 41.9–66.1%). They concluded that lifetime cancer risks may be lower for CRC and endometrial

cancer than presently assumed and LS should be considered in older patients, although this data must still be confirmed. On the whole, the earlier-described data show that the lifetime risks are similar to or lower than previously published estimates and the age-specific penetrance of these mutations is considerably lower than previously thought. In the literature, age at onset of LS almost invariably is referred to as 44–45 years, sometimes with ranges of 42–48 years [25, 36–38]. Lifetime risk estimates from similar studies with varying methods of ascertainment have appeared in several recent publications. Data suggest that limiting molecular studies to patients with an early age at diagnosis will miss many cases in the diagnosis of LS.

17.3 Endometrium

An estimated 5% of all cases of EC are associated with a hereditary cause [39]. EC is the most common LS-associated extracolonic tumor. In a study by Watson et al. [20], the cumulative incidence of LS-associated EC was approximately 20% by age 70, compared to 3% in the general population. Risk is highest in women most likely to carry the LS gene. In this group, during the highest risk years (age 40–60), average annual risk exceeded 1%. Some MMR mutations in female carriers increased the risk of EC to 40–50%.

In more than 50% of women with LS, EC is the first, or "sentinel," cancer to develop [40]. Like in CRC, the age of onset for EC in LS is significantly younger than the average age of onset of EC in nonmutation carriers [41]. Studies of women with LS have found a mean age at diagnosis of 48–49 years for EC [33, 42] and 42 years for ovarian cancer [43]; and most cancers were diagnosed in women over age 35. Like in CRC, high levels of microsatellite instability (MSI-high) in EC can result from germline mutation of the DNA MMR MLH1, MSH2, or MSH6 or, in the sporadic setting, from methylation of MLH1 [44].

Schmeler et al. [45] studied 315 women with LS. In this group, 107 women (34%) were diagnosed as having CRC. Forty-one patients had synchronous (three patients) or metachronous (38 patients) CRC and endometrial or ovarian cancer (32 and 9 patients, respectively). The median age at diagnosis of CRC was 47 years (range, 26–77). Five of the 41 women (12%) were 35 years old or younger, six (15%) were 36–40 years of age, five (12%) were 41–45 years of age, and 25 (61%) over age 45. Twenty-one of these 41 women (51%) received a diagnosis of gynecologic cancer after receiving a diagnosis of and undergoing surgery for CRC. The median time between the diagnoses of CRC and gynecologic cancer was 5 years (range, 1–25). One hundred thirty-seven (43.5%) cases presented mutations in hMLH1, 174 (55.2%) in hMSH2, 3 (0.9%) in hMSH6 and 1 case (0.3%) presented mutations in both MLH1 and MSH2. This study provides evidence of a benefit of prophylactic hysterectomy and bilateral salpingo-oophorectomy in preventing gynecologic cancers in women with LS.

In Oliveira Ferreira et al. [27] study, of 29 families with LS, 201 patients with cancer were identified among 1,241 individuals (589 men and 652 women). In these 201 patients, 223 tumors were observed, being 137 CRCs (55 in men and 82 in women) and 86 extracolonic tumors (37 in men and 49 in women). The more frequent extracolonic primary sites in women were endometrium (26.5%) and breast (26.5%). Twenty-one patients presented a second primary tumor: CRC in seven cases, endometrium in four, breast in three, stomach in two, ovary in two, hepatobiliary tract in two and prostate in one. One patient presented three different primary tumors during life: colon cancer at age 26, endometrial at age 31, and sebaceous carcinoma also at age 31.

Patients with LS have a better prognosis than patients with common sporadic CRC. Boks et al. [42] compared survival rates of LS-associated EC with sporadic EC. The outcomes in survival in EC in the general population and in women from families with LS did not differ significantly (overall 5-year cumulative survival rates: 82% vs. 88%). Besides, there was no significant difference in the distribution of tumor histological subtypes in the study group compared to the control group.

In the general population, about 80% of sporadic ECs are endometrioid, while about 20% are nonendometrioid [46, 47]. The pathological features of EC in LS have been studied in a few series. Parc et al. [48] showed that EC with MSI is associated with FIGO stage and grade, cribriform growth pattern, mucinous differentiation and necrosis. Broaddus et al. [49] analyzed 50 women with LS and EC from four different hereditary cancer datasets. The results were compared with those of two different groups of sporadic EC women younger than age 50 ($n=42$) and women of all ages with tumors presenting MSI-high secondary to methylation of MLH1 ($n=26$). They found 78% endometrioid and 22% nonendometrioid EC, a result very similar to the spectrum of tumor histologies seen in the general population. Nearly one-fourth of LS patients in the study had endometrial tumors with pathologic features that would require adjuvant therapy after hysterectomy (22% were Stage II or Stage III). Besides, 14% of LS-associated endometrial tumors were classified as a histotype associated with a more aggressive clinical course. There was a trend toward the LS patients, carriers of MSH2 mutations, having more nonendometrioid tumors. Such nonendometrioid tumors were extremely rare in the MLH1 methylated group. A subset of MLH1 methylated sporadic tumors showed a unique, "undifferentiated" histology that was not observed in LS or the younger group. Data suggest a genotype–phenotype relationship in which microsatellite instability resulting from MLH1 methylation is associated almost exclusively with classical or "undifferentiated" endometrioid tumors, whereas microsatellite instability secondary to MSH2 mutation can result in an EC more variable histological spectrum.

It is known that about 29% of women with complex atypical hyperplasia (CAH) detected on endometrial biopsy will progress to EC [50]. During the collection of cancer cases for the LS group, Broaddus et al. [49] found two cases of CAH in hysterectomy specimens; both had endometrial endometrioid adenocarcinoma, Grade 1, associated with complex hyperplasia. Neither tumor was invasive. According to the authors, in the general population many low-grade endometrioid adenocarcinomas are derived from the precursor lesion CAH. Limited information at this time suggests that CAH is indeed a part of the pathogenesis of endometrioid tumors in LS.

17.4 Urinary Tract

Sijmons et al. [51] studied the risk of the different types of urinary tract cancer in LS families and reviewed screening options. They retrospectively calculated the relative and cumulative risks of developing urinary tract cancer by comparing tumor occurrence in patients and their first degree relatives in the Dutch hereditary HNPCC registry with those in the general Dutch population. A person–year analysis was used, including data on 1,321 individuals from 50 hereditary HNPCC families. The relative risk of developing transitional cell cancer of the renal pelvis or ureter was 14.04 (95% confidence interval, 6.69–29.45, $p<0.05$) and the cumulative risk was 2.6%. The risks of renal (excluding renal pelvis) and bladder cancers were not significantly increased. Urinary tract cancer was diagnosed at a relatively young age and many women were affected. Some familial clustering was observed. These findings indicate that LS is associated with an increased risk of transitional cell cancer of the upper urinary tract. The cumulative risk is relatively low, although a subset of LS families may be exposed to a much higher risk.

17.5 Small Bowel

Adenocarcinoma of the small bowel (SBC) is rare and accounts for only about 1% of all gastrointestinal tract cancer. LS patients are at increased risk of small bowel carcinoma. In 1985, Love [12] reported a patient with small bowel adenocarcinoma in an LS kindred. Later, Vasen et al. [52] and Lynch et al. [53] reported small bowel adenocarcinoma association with LS. Lynch et al. [53] reported small bowel adenocarcinoma in nine patients from eight LS extended pedigrees. Each affected patient was in the direct genetic lineage or manifested multiple primary cancers (stomach, colon, endometrium, and ovary) consonant with the tumor spectrum of LS. The average age of onset for small bowel cancer was 47 years (range 31–56 years), compared to the general population peak occurrence after the sixth decade. They concluded that small bowel cancer may be an integral component of the tumor spectrum of LS.

Rodriguez-Bigas et al. [54], studying clinicopathological data in their registries of the *International Collaborative Group on HNPCC* (ICG-HNPCC), found 42 individuals from 40 LS families that developed 42 primary and seven metachronous small bowel tumors. There were 46 adenocarcinomas and three carcinoid tumors. The median age at diagnosis of the index small bowel tumor was 49 years. Although the most common site of the primary tumor was the duodenum (36%), the tumors were nearly evenly distributed throughout the small bowel. MMR gene mutations were present in 15 of 42 patients (36%). There were nine hMLH1 and six hMSH2 mutations. The small bowel was the first site of carcinoma in 24 patients (57%). The median survival for the 42 patients was 47 months (range, 0–447 months). The overall 5- and 10-year

survival rates were 44% and 33%, respectively. Compared to the general population, small bowel adenocarcinomas in LS patients occur at an earlier age and appear to have a better prognosis. In Rodriguez-Bigas et al. [54] study, the median age at diagnosis of small bowel carcinoma was 49 years, which is approximately 19 years younger than the median age at diagnosis of small bowel carcinoma in the general population [55]. The male to female ratio was 3:1, which is twice that of the reported in SEER population-based tumor registry studies for adenocarcinoma of the small bowel [56].

Schulmann et al. [57] analyzed the features of small bowel cancer in LS in 31 unrelated patients with 32 SBCs (one patient had two synchronous SBCs). Median age at diagnosis was 39 years (only one SBC was diagnosed before age 30–15 years). Twenty-two patients (69%) were men. Fifty percent of SBCs were located in the duodenum, with a decreasing frequency from the duodenum to the ileum. The Amsterdam criteria were fulfilled in 50% of patients; 15 patients met at least one of the classic Bethesda criteria 2, 3, or 4; 45% of patients had no personal history of previous malignancies. Two patients (6%) had a positive family history for SBC. SBC was part of the first clinical manifestation of LS in 14 patients (45%); SBC was the only site of malignancy in six patients. In four patients, SBC was the first neoplasm, later followed by other LS-related malignancies during follow-up. In four cases, SBC was synchronously detected with other LS-related malignancies (all were CRC) as the first manifestation of disease. Seventeen patients (55%) had a history of previous LS-related malignancies before diagnosis of neoplasm of the small bowel; in 14 of 17 cases, the previous diagnosis was CRC. There were 24 adenocarcinomas, one adenoma, and one carcinoid tumor (seven tumors were not classified histologically). One must remark that the carcinoid tumor displayed MSI-H and loss of MLH1 expression and occurred in a carrier of an MLH1 germline mutation. Miquel et al. [58] also reported an unusual case of a 28-year-old woman with LS who underwent surgery for a transverse colon adenocarcinoma in whom an appendix carcinoid tumor was incidentally found and showed that both tumors had normal expression of the MMR proteins hMLH1, hMSH2, hMSH6, and hPMS2 (the adenocarcinoma exhibited an MSI phenotype but the carcinoid tumor did not). In Schulmann et al. series, there were five T2, 11 T3, and 10 T4 tumors. Seventeen tumors had no lymph node metastasis, whereas eight presented them (seven had N1 and one had N2). Five patients had died after a median follow-up of 48 months after SBC being detected. Two patients died from SBC, two patients from other metachronous cancers, and one died from postoperative complications. The overall 10-year survival rate was 87%. In patients with regional or disseminated disease and sufficient follow-up information (24 months), two of five patients (40%) died from SBC. In contrast, none of 16 patients having localized disease and a minimal follow-up of 24 months died from SBC. Pathogenic germline mutations were identified in 81%; high MSI was detected in 95% and loss of MMR protein expression in 89% of cases. An expansive growth pattern of the tumor border and an intense intratumoral lymphocytic infiltrate were present in 75% of cases.

17.6 Brain

Different studies reported an association between LS and brain tumors. Vasen et al. [59] used risk analysis to compare families with LS to those in the general population. Of the 1,321 subjects from 50 LS families (with 60,237 person-years of follow-up) in the Dutch HNPCC Registry, which satisfy the Amsterdam Criteria, 312 had CRC. The registry showed 14 brain tumors in the LS-patients and their first-degree relatives: five astrocytomas, three oligodendrogliomas, one ependymoma and five tumors for which a pathological report was not available. The relative risk of having brain tumors in patients with LS and their first-degree relatives was six times greater than in the general population (95% confidence interval, 3.5–10.1). After excluding cases based only on family history, the relative risk was 4.3 (95% confidence interval, 2.3–8.0). Although an increased relative risk of brain tumors was found, the lifetime risk was low (3.35%). As it is not certain whether an improvement of the overall prognosis can be achieved by early diagnosis and intervention, and considering the low lifetime risk, screening for brain tumors in LS families is not recommended.

17.7 Stomach

There is some evidence suggesting that gastric carcinoma is the second most common LS-associated extracolonic malignancy. According to a series of reports on family G, the first LS family described, gastric cancer was the most common tumor at the time of the initial description – when the incidence of gastric cancer was extremely high in the background population. From then on, the incidence of gastric cancer in family G decreased rapidly in accordance with its decreasing incidence in the background population [60, 61]. This dramatic change in family G's extracolonic cancer spectrum, mainly the decline in gastric cancer incidence, strongly implies that LS phenotype, including the frequently associated extracolonic cancer, may vary according to the cancer spectrum of the general population.

The cumulative risk of stomach carcinoma in putative LS gene carriers has been estimated at 19%. Gastric carcinoma manifests at younger ages in LS than it does in sporadic cases. Aarnio et al. [62] examined the features of gastric cancer in LS. The frequency of gastric cancer was 11% among putative gene carriers. The mean age of the 45 gastric carcinoma patients (24 men and 21 women) was 56 years (range 31–85 years). Other metachronous cancers occurred in 23 cases (51%). The total number of tumors was 90. Gastric tumor was the only tumor in 22 patients and the first malignancy in five others. Eighteen patients (40%) had already been treated for CRC (13 cases), endometrial cancer (2 cases), ovarian cancer (1 case), urinary tract cancer (1 case) and testicular cancer (1 case). A variation from zero to 40% (mean 11%) in the occurrence of gastric cancer was observed in families with different mutations in MLH1 or MSH2 genes. Nineteen tumors (79%) showed features of the intestinal type of gastric cancer; six (32%) were poorly differentiated;

four others (17%) were classified as diffuse (3) or mucinous (1). Seventeen (38%) were in the proximal third of the stomach; seven (16%) in the medium third; and 19 (42%) in the distal. The histological distribution differs from the usual pattern in Finland (51% intestinal type and 37% diffuse type). This difference might be due to the fact that the diffuse type predominates in young patients and the intestinal type is almost absent in those under 60 years of age [63, 64] (in Aarnio et al. [62] study, 11 patients, 58%, with the intestinal type of cancer was younger than 60 years old). Diffuse-type histology is characteristic of familial gastric cancer associated with the E-cadherin mutation [65], whereas the intestinal type is far more prominent than the diffuse type in gastric cancers caused by a mutation of the MMR gene [62]. Exact information on the histological type of a given gastric cancer would be very helpful in discriminating between gastric cancer caused by the MMR gene mutation and gastric cancers with other etiology.

In Korea, an endemic area for gastric cancer, Park et al. [26] investigated 1,011 individuals from 66 Korean LS families (28 families fulfilled the Amsterdam criteria and 38 did not). Twenty-five patients with gastric cancer were identified among 22 LS families. The risk of gastric cancer in patients with LS and their first-degree relatives was 2.1-fold greater than in the general population (95% confidence interval; range, 1.4–3.2). But the relative risk of gastric cancer in the younger generations was much greater (11.3-fold in the 30s and 5.5-fold in the 40s). Additionally, the relative risk was greater in mutation-carrier families than in noncarrier families (3.2-fold vs. 1.6-fold). This study demonstrated that the risk of gastric cancer in members of LS families in a gastric cancer endemic population, particularly in younger subjects and mutation carriers, is high enough to justify careful screening.

In Brazil, where the prevalence of gastric cancer is also high, in the 29 families of LS studied, 10.2% of gastric cancer in women and 35.1% in men [27] were found. In Chinese LS patients, gastric cancer occurred more frequently, accounting for 11.9% of all cancer patients and ranking second in the spectrum of LS-predisposing cancers [66].

17.8 Hepatobiliary Tract and Pancreas

Mecklin et al. [67] evaluated 18 patients with a biliopancreatic carcinoma in 15 different *cancer family syndrome* (CFS) families. In 11 (79%), the tumor was confirmed as a carcinoma of the biliary tract or papilla of Vater. In three (21%), carcinoma of the pancreas was the most probable alternative. In all four patients without histologic reevaluation, the diagnosis was carcinoma of the biliary tract. Vernez et al. [68] reported a case of Muir–Torre syndrome associated with intrahepatic cholangiocarcinoma, with a novel missense mutation of the MSH2 gene (c.2026T>C).

Banville et al. [69] described the case of a patient with CRC e pancreatic medullary carcinoma that presented germinative mutation in hMSH2 gene. In addition, the tumor showed microsatellite instability and loss of expression of the mismatch repair proteins MSH2 and MSH6.

17.9 Skin

Sebaceous glands neoplasias comprise adenomas, epitheliomas, and carcinomas. They are a characteristic manifestation of Muir–Torre syndrome (MTS), a phenotypic variant of LS [70]. MTS diagnosis is done by at least a sebaceous neoplasia and at least an internal neoplasm in the same patient, regardless of family history [71].

There is a very strong association between sebaceous neoplasias and CRC, with a trend of association with the same extracolonic tumors pertaining to the spectrum of LS tumors [72]. As a phenotypic variant of LS, MTS also is strongly related to MMR genes, mainly hMSH2, and microsatellite instability. Mangold et al. [70] analyzing 41 patients from different MTS families demonstrated loss of DNA MMR protein expression and/or high microsatellite instability. There were detected germline mutations in 27 of the 41 patients (66%), and 16 of them were described for the first time as related to MTS. Twenty-five mutations (93%) in the MMR genes were predicted to lead to a truncated protein. Most mutations occurred in gene HMSH2 (25/27 – 93%).

Kruse et al. [73] studying 16 patients with MTS, all carriers of sebaceous neoplasia and CRC, demonstrated that all patients presented high genomic instability in at least one of the two tumors. Thirteen of the 16 patients had been evaluated in relation to mutations in the genes hMLH1 and hMSH2. Nine (69%) presented truncating mutations, eight of them in hMSH2.

Therefore, sebaceous neoplasias are part of the spectrum of LS tumors, inside the phenopypical variant of MTS. Because of its rarity, whenever the diagnosis of a sebaceous neoplasia is done, one must suspect LS/MTS.

17.10 Breast

Breast cancer occurs with a relatively high frequency in the general population, but the inclusion of breast cancer as part of LS is controversial. Since the discovery of mutant MMR genes and the corresponding microsatellite instability, a large number of studies have documented microsatellite instability in many sporadic tumor types, including those not associated with LS [74], but MMR gene mutations in the sporadic tumors with microsatellite instability phenotype are rare. Many studies have also quantified the microsatellite instability phenotype in breast cancer, with an incidence ranging from zero to 20% of tumors studied [75, 76]. Microsatellite instability is also present in 10–20% of all sporadic breast cancer cases, and is thus a nonspecific test [77]. In Contegiacomo et al. [78] study, microsatellite instability significantly correlated with the lobular histotype and also with lymph node involvement. A trend was also observed that associates microsatellite instability and large tumor size. Thus, microsatellite instability functions as a marker for inherent susceptibility for developing cancer, and it has been observed in different types of LS-related cancer types [79].

Some studies suggest that the previous history of breast cancer constitutes a risk factor to the development of CRC, however, other studies do not confirm the association [80]. Some hypotheses are suggested, most of which focus on the possibility of a genetic predisposition and epidemiologic characteristics linked to exposure to environmental agents. Amanti et al. [81] had evaluated 71 operated breast cancer patients through colonoscopy. Three patients (4.2%) presented history of CRC and 18.3% (13 cases) presented intestinal polyps. Ninety-three percent of patients had a relative with a neoplasia history. A cohort study demonstrated that women with breast cancer above of 65 years of age present an increased risk of development of colorectal adenomas when compared to women without breast cancer [82]. Another study demonstrated a risk two-and-a-half times higher for the development of neoplastic injuries and adenomatous polyps for patients previously treated for breast cancer [83]. In a control group case study, the incidence of CRC was associated to a familiar history of breast cancer (OR = 2.1, IC 95% 1.1–4.1) [84]. Other authors had also shown a correlation between both neoplasias [85].

The frequency of adenomatous polyps and CRC was studied in 95 mastectomized breast cancer patients, and the prevalence of breast cancer was evaluated in 77 women previously operated for colorectal cancer. The frequency of adenomatous polyps and adenocarcinoma among mastectomized women was 10.5% and 5.3% and among the control group (NS), it was 8.5% and 3.9%, respectively. However, the prevalence of breast cancer among women treated for CRC was of 5.2% (cases) and 0.3% (controls), ten times higher in the first group. The findings are consistent with the hypothesis of a correlation between breast cancer and CRC [86]. Other studies, however, had not demonstrated a positive association [87, 88]. The results are conflicting, but part of the publications suggests an increased risk for development of polyps and CRC in patients treated for breast cancer. Also the women treated for CRC seem to have an increased risk of developing breast cancer.

Family history of breast cancer is an established risk factor for this disease and is used to identify women at higher risk, although the impact of risk factors for breast cancer among women with a family history is not well defined. In the USA, the percentage of breast cancer women with a family history of breast cancer varies from 6% to 19% [89], and most of them are associated with mutations in the genes BRCA1 and BRCA2, but some authors think that an undetermined number may be associated to LS [90].

The cumulative lifetime risk of breast cancer for women that carry the susceptibility allele is predicted to be high, about 92%, while the cumulative lifetime risk for noncarriers is estimated to be about 10% [91]. Colditz et al. [92] analyzed data obtained prospectively from the Nurses' Health Study. Among women with a family history of breast cancer, reproductive risk factors had associations different from those observed among women without a family history of the disease. They observed a consistent increase in breast cancer risk among women with a mother or sister history of the disease that was exacerbated by first pregnancy.

Oliveira Ferreira et al. [27], in Brazil, demonstrated that breast cancer and EC were the most frequent extracolonic tumors in women from LS families (26.5% each). Thirteen cases of breast cancer were observed in 652 women from LS

families. High frequencies of gastric cancer and EC were expected, since those tumors are part of the LS spectrum. The cause of this fact is not known. The high frequency of breast cancer observed in the studied families requires further molecular investigation to determine a possible hereditary correlation. At this point, there is not an specific breast cancer screening for LS families in Brazil.

One knows that hereditary breast cancer is associated to alterations in the BRCA1 or BRCA2 genes, and that the risk of CRC in patients with BRCA1 mutation is 4.1%, increasing to 6% after age 70, compared to a 1–2% risk in the whole population [25].

Several reports offer conflicting data as to whether LS family members are at increased risk for breast cancer [6, 17, 93]. Itoh et al. [94] analyzed 130 suspicious pedigrees of Lynch Syndrome to estimate the relative risks of cancer in first-degree relatives of index patients. The risk of death from all causes was significantly increased in women over 45 years of age and the overall liability to cancer in women was greater than for men. A sevenfold increase in CRC risk was found in both sexes. In female relatives, the risk of breast cancer increased fivefold and lifetime risk of breast cancer was one in 3.7. Nelson et al. [95] analyzed data from the Iowa Women's Health Study (IWHS), a population-based sample of 41,837 women aged 55–69 years, to determine if the familial clustering of malignancies related to LS is more common in women with cancer than without. Self-reported information was collected on history of colon, uterine, ovarian, and breast cancers in female first-degree relatives. A family history of breast cancer (odds ratio [OR] = 1.4), colon (OR = 1.3) and uterus cancer (OR = 1.3), but not ovary cancer (OR = 1.2), was significantly more common among women with a personal history of any of these four cancers (all $p < 0.05$); the pattern of the ORs strongly suggested that the clustering tended to be site-specific. Age-adjusted relative risks (RR) of incident CRC over 5 years of follow-up ($N = 237$) were calculated with regard to family history. CRC incidence was increased among women with a family history of breast (RR = 1.3), uterine (RR = 1.4), colon (RR = 1.5), and ovarian (RR = 1.3) cancers, although none of the risk estimates achieved statistical significance. RR was, however, significantly related to the number of different cancer sites reported among family members (Ptrend = 0.008). The authors concluded that these data on a representative sample of postmenopausal women suggest that family histories of colon, breast, uterine, and ovarian cancers are associated with an increased risk of cancer at the same site, but provide little support for the hypothesis that LS is a nonrandom occurrence. Houlston et al. [96] in a screening program for first-degree relatives of CRC patients (relatives of patients who had developed CRC before age 45 and members of families in which multiple cancers had occurred), found 62 cases with polyps and 5 cases with colorectal cancer from 382 high risk individuals submitted to colonoscopy. Women with family histories compatible with LS were offered screening for breast and pelvic tumors. One hundred and ten pedigrees were identified with the LS, and four of 35 women screened were found to have breast cancer.

Risinger et al. [90] carried out a mutation screening of MMR genes in breast tumor tissue in which a 4-base pair frameshift mutation in the hMLH1 gene was

found to segregate with disease in the germlines of the affected members of a large LS kindred. Expression of only the mutant allele was observed in the breast cancer tissue of one family member; however, both alleles were observed in her normal tissue. This breast cancer exhibited widespread microsatellite instability, as did breast cancers obtained from several other LS kindred. These data indicate that breast cancer may result from an inherited mutant MMR gene and that breast cancer may occur as an integral tumor in the LS syndrome. As discussed in Risinger et al. [90] study, data suggest that breast cancer occurring in the context of an unknown fraction of LS patients and families may result from inheritance of a mutant MMR gene, thus representing another manifestation of hereditary breast cancer.

Westenend et al. [97] studied a 49-year-old woman, member of an LS family with breast cancer. They showed she had a 2-base pair deletion in exon 11 of the MMR gene MSH2 (c1705_1706 delGA). Microsatellite analysis of the tumor showed a microsatellite instable pattern for markers Bat25, Bat26, and Bat40. Immunohistochemical staining for the mismatch repair enzymes MSH2 and MSH6 was negative, whereas the tumor cells were positive for MLH1, a pattern suggestive of biallelic MSH2 gene inactivation. Testing the tumor for loss of heterozygosity of the MSH2 gene, they found loss of the wild-type MSH2 allele. Data strongly suggest that the MSH2 gene was involved in the development of this breast tumor. Boyd et al. [98] identified a male member of a large LS kindred, affected by primary malignancies of the breast and colon. This individual was found to harbor a germline mutation of the MLH1 MMR gene previously shown to segregate with disease in this kindred. The breast tumor exhibited somatic reduction to homozygosity for the MLH1 mutation, and microsatellite instability was evident in it. They conclude that hereditary male breast cancer can occur as an integral tumor in the LS syndrome.

Borg et al. [99] have identified hMLH1mutations in two Amsterdam-criteria LS families where both male and female gene carriers had breast cancer. They analyzed the two breast cancer susceptibility genes BRCA1 and BRCA2. In one family, they did not find any mutation in the breast cancer genes, while in the other, a BRCA1 mutation segregated in the breast cancer cases. The tumor from a woman with both hMLH1 mutations and a BRCA1 mutation exhibited typical BRCA1 histology, for example, grade 3 invasive ductal carcinoma with dense lymphocytic infiltration, and immunohistology, estrogen receptor (ER) negative, progesterone receptor (PgR) negative, strongly p53 positive, c-erbB-2 negative, and highly Ki67 positive (>50% stained cells). The histology of the breast tumor from the man with both one hMLH1 mutation and a BRCA1 mutation was a grade 2 invasive ductal carcinoma without any special BRCA1 features. This might merely reflect a true difference in male breast tumor progression versus female. They could not exclude that the combined effect of BRCA1 and hMLH1 dysfunction has a bearing on tumor progression.

Based on these data, there is a suggestion that MMR gene mutations may cause hereditary breast cancer and that breast cancer represents an integral tumor in the LS.

Table 17.3 Lifetime risk and age of diagnosis of the main extracolonic tumors in LS patients

Extracolonic tumor	Mean age at diagnosis (years)	Lifetime risk of extracolonic cancers	Relative risk of tumour LS patients	Histology
Endometrial	62.0 [35] 48–49 [33, 42]	20% by age 70 [20] 40%–60% [5, 7, 29]	–	Endometrioid 78%; nonendometrioid 22% [49]
	MMR Mutations: 40–60 [20]	54% [35] hMLH1 Mutation carriers: 42% [30] hMSH2 Mutation carriers: 61% [30] hMSH6 Mutation carriers: 71% by age 70 [32]		
Ureter/renal pelvis	hMLH1 Mutation carriers: 63 (52–72) [33] hMSH2 Mutation carriers: 56 (40–72) [33]	2.6%–10% [5, 7, 29] 2.6% [51]	22/3.2 [6] hMSH2 Mutation carriers: 75.3 (31.3–180.9) [30] 14.04 [51]	Transitional cell carcinomas [51]
Small bowel	39 [57] 47 (31–56) [53] 49 [54] hMLH1 Mutation carriers: 50 (25–67) [33] hMSH2 Mutation carriers: 51 (31–69) [33]	–	25 [6] hMSH2 Mutation carriers: 102.6 (14.5–728.6) [30]	Adenocarcinoma decreasing frequency from the duodenum to the ileum [54, 57]
Stomach	56 (31–85) [62] hMLH1 Mutation carriers: 53 (39–74) [33] hMSH2 Mutation carriers: 51 (23–82) [33]	19% [5, 7, 29]	4.1[6] hMSH2 Mutation carriers: 19.3 (6.2–59.9) [30] 2.1; 11.3 (in the 30s); 5.5 (in the 40s); mutation carrier = 3.2 [26]	79% intestinal type [62]

Ovary	42 [43] hMLH1 Mutation carriers: 51 (35–75) [33] hMSH2 Mutation carriers: 45 (37–58) [33]	10%–12% [5, 7, 29]	3.5 [6] hMSH2 Mutation carriers: 7.97 (1.1–56.6) [30]	–
Brain	hMLH1 Mutation carriers: 45 (21–78) [33] hMSH2 Mutation carriers: 41 (2–73) [33]	3.35% [59]	4.3 [59]	Astrocytoma, oligodendroglioma, ependymoma [59]
Hepatobiliary tract	–	17.5% [7]	–	–
Skin (sebaceous tumors)	–	–	–	Adenomas, ephiteliomas and carcinomas, keratoacanthomas [70]
hMLH1 Mutation carriers	–	11% [31]	–	–
hMSH2 Mutation carriers	Males: 55.4; Females: 39.0 [31]	48% [31] Males: 34%; Females: 69% [31]	–	–

Probably the penetrance of this genetic defect in causing breast cancer must be lower than that for CRC or EC, depending upon genetic and environmental modifiers [90].

Wang et al. [100] evaluated the involvement of the various MMR genes in typical and incomplete LS syndromes. They suggest that the presence of multiple primary malignancies in a single individual and the observation of extracolonic tumors in relatives of a CRC patient should be included among the guidelines for referring patients for genetic testing.

17.11 Conclusion

The constant progress observed in LS clinical and molecular characterization suggests a dynamic interpretation of the facts (Table 17.3). Thus, the possibility of changes in the criteria used for diagnosis can be foreseen. In this sense, a definition of the spectrum of extracolonic tumors as invariant certainly would cause interpretation errors. Frequent new molecular findings have been of utmost importance for a better definition of the genotype–phenotype correlation and thus of the spectrum of extracolonic tumors in LS.

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Chapter 18
Surveillance

Heikki J. Järvinen and Jukka-Pekka Mecklin

Abstract In Lynch syndrome surveillance aims at prevention of early detection of cancer types observed to occur in significant excess. At present, regular prophylactic examinations have been shown beneficial for colorectal and endometrial cancer, i.e. repeated colonoscopy and endometrial biopsy combined with ultrasonograpy. For other less common cancer types involved, no proven benefit has been shown. The guidelines of identifying Lynch syndrome, the principles of genetic testing, and arranging surveillance are reviewed in this article, also shortly dealing with prophylactic surgery and possible chemoprevention in future.

Keywords Colonoscopy • Colorectal carcinoma • Endometrial carcinoma • Lynch syndrome • Cancer prevention • Polypectomy • Endometrial biopsy • Prophylactic surgery

18.1 Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome provides an exceptionally good opportunity for early detection and prevention of the predominant cancer types involved. This is based on the observations of very high cumulative lifetime incidences of colorectal (60–100%) and of endometrial cancer (40–60%) in subjects harboring a pathogenic mismatch repair gene mutation [1–3]. The possibilities of preventive measures against many other tumor types with a moderately increased life-time cancer risk of around 10% or less [1–3] remains less clear, especially as long-term experience on prospective follow-up studies on

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Table 18.1 Principles of early disease detection by Wilson and Junger [4]

1. The condition sought is an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not "once and for all" project.

mutation-positive subjects is still limited and surveillance trials are few. The latter tumor types include, at least, gastric cancer, ovarian, bile duct, uroepithelial and kidney and small bowel cancers, and brain tumors.

In considering the usefulness of surveillance programs in HNPCC, the general principles in disease screening developed by Wilson and Junger in 1968 [4] may be used as guidelines (Table 18.1). While these principles of secondary prevention were originally aimed at mass screening of large populations to detect common health problems such as tuberculosis, diabetes, hypertension and some cancers, they are well suited for even more limited and defined high-risk groups, such as HNPCC covering from 1 to 5% of all new cases of colorectal cancer [5, 6]. The beneficial effect of surveillance in the context of a dominantly inherited cancer condition is accentuated by the facts that the risk involves young age groups enabling saving of many years of life in each case and that every high-risk person identified brings several other high-risk family members into the reach of cancer prevention program. The present review shortly describes some basic principles of identification, genetic testing, and surveillance recommendations in families with HNPCC.

18.2 Identification of HNPCC

The key for the identification of HNPCC is a careful family history in each new case of colorectal cancer. In addition to the occurrence of other cases of colorectal cancer in close relatives, early age of onset, proximal site of the tumor, and the presence of multiple tumors (synchronous or metachronous) should arise suspicion. Before genetic diagnostic tests, the use of some uniform diagnostic criteria such as the Amsterdam criteria II (Table 18.2) or Bethesda criteria (Table 18.3) may be helpful even though none of the clinical features are diagnostic or exclusive [7, 8]. The Bethesda criteria are more sensitive to identify HNPCC cases for genetic analyses. Even more accurate may be to use computed models for the risk analysis for the selection into further study [9]. It should be remembered that other tumors

Table 18.2 Amsterdam criteria II [7]

1. At least three relatives with colorectal cancer or other Lynch syndrome-associated cancer (endometrial, small bowel, urothelial)
2. One relative with cancer should be a first-degree relative with the other two
3. At least two successive generations should be affected
4. At least one of the cancer patients should be <50 years of age at diagnosis
5. Familial adenomatous polyposis should be excluded
6. Tumors should be verified by histopathology

Table 18.3 The revised Bethesda criteria [8]

1. Colorectal cancer diagnosed in a patient <50 years of age
2. Presence of synchronous, metachronous colorectal, or other Lynch syndrome-related tumors[a] regardless of age
3. Colorectal cancer with MSI-high phenotype diagnosed in a patient <60 years of age
4. Patient with colorectal cancer and a first-degree relative with a Lynch syndrome-related tumor, with one of the cancers diagnosed <age 50 years
5. Patient with colorectal cancer with two or more first-degree or second-degree relatives with a Lynch syndrome-related tumor, regardless of age

[a]Lynch syndrome-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter, renal pelvis, biliary tract, and brain tumors, sebaceous gland adenomas and keratoacanthomas, and carcinoma of the small bowel

besides colorectal cancer might present as the index tumor of an HNPCC family such as endometrial, urothelial, or small bowel cancer.

When mismatch repair gene mutation is suspected, the tumor should be examined for microsatellite instability (MSI), which is present in most HNPCC-associated colorectal cancers. Another approach is to use immunohistochemical staining of the tumor to show MMR-gene protein expression. A negative staining directs further mutation search to the probably causative gene (MLH1, MSH2, MSH6 or PMS2). Because immunohistochemistry is slightly less sensitive than the MSI test, both approaches can be used simultaneously for higher accuracy [10]. Final diagnosis of HNPCC will be achieved only by demonstration of a pathogenic germline mutation in a mismatch repair gene in normal tissue by sequencing. This is possible in some 80% of clinically suspicious families depending on criteria used.

It is important to note that surveillance is most effective in families with a known mutation as mutation-negative family members can be omitted from surveillance. Naturally, surveillance can be practiced even with no mutation data but in that case half of the at-risk persons will undergo unnecessarily repeated colonoscopies with all the inconvenience and risks connected with it.

18.3 Genetic Testing

A definite identification of a pathogenic germline mutation in one of the mismatch repair genes makes predictive genetic testing of unaffected family members possible. Testing should be offered for all first-degree relatives of the affected person,

that is, siblings, children, and parents. Drawing of the family tree greatly helps to decide to whom testing should be extended depending on the occurrence of cancer in the family.

Thorough personal genetic counseling should always precede the testing. The information should include the genetic nature of the disorder, type of the inheritance, the magnitude of the various cancer risks involved, and the clinical management strategies of prevention. The estimated success of early detection or prevention should be given as well as the fact that for all cancer types involved no surveillance is available. The counseled must realize before testing that knowledge of the mutation status may cause anxiety and that a mutation-positive finding necessitates a life-long endoscopic surveillance program or other appropriate preventive measures. The potential risk of limitations or discrimination caused by the test result, for example, by insurance companies or employers, must also be discussed. For these reasons, testing and genetic counseling is not advised for children until the age of 18–20 years when prophylactic surveillance becomes actual. Personal written informed consent is recommended before testing and subsequent registration for surveillance of the mutation-positive subjects.

The present views of optimal surveillance are still under development and new types of intervention may become available in future. After about 12 years of practical experience on surveillance based on mutation testing knowledge about long-term efficacy in cancer prevention and about consequences on quality of life and psychological well-being are still limited.

Integration of the genetic testing with the organization of surveillance is an important issue. As members of a single family often live widely scattered around the country, a centralized registry is essential with permanent data records containing information about the test results, surveillance visits and possible cancer incidence and treatment. The practice of organizing registration and surveillance may vary in different countries, but in general, nationwide or at least large regional registries are optimal even though surveillance visits and treatment can be directed to local health organization. In a genetic cancer predisposition, it is most important that a permanent availability for genetic testing and surveillance can be guaranteed for the HNPCC families from generation to generation once the underlying predisposition has been diagnosed. The economic burden of the testing and surveillance may also distribute variably in different countries. In some countries, the public health system takes care of all costs, in others they belong to private health insurance or for the persons themselves.

The acceptance rate of genetic testing has varied widely from 43 to 75% of risk subjects completing the counseling and choosing testing [11, 12]. In the Finnish experience, with over more than 10 years of surveillance on more than 660 mutation-positive subjects, the compliance rate has been very high approaching 98% in keeping within the surveillance program [13]. The registry organization has a great responsibility to maintain high compliance by creating good contacts with the HNPCC families.

18.4 Screening Guidelines

18.4.1 Colonoscopy Surveillance

The idea of repeated colonoscopies in HNPCC is based on the assumption that despite the misleading name "nonpolyposis colorectal cancer," the cancer is always preceded by a detectable precancerous lesion, an adenomatous polyp, or at least a flat adenomatous dysplastic change, even in the context of HNPCC. It has been suggested that the number of adenomas in mutation-positive subjects is not necessarily much higher than in normal population, but the progression to carcinoma is exceptionally rapid [14]. Therefore, endoscopic detection and removal of all polyps would provide an effective means to prevent cancer or, at least, allow an early diagnosis of cancer in an asymptomatic phase resulting very likely in an excellent prognosis.

In one study, including 360 mutation-positive subjects or obligate mutation carriers from 50 separate families, excluding probands of each family, the overall probability of colorectal cancer by the age of 70 years was estimated to 80%, 54% for women and nearly 100% for men [3]. Some more recent estimates have suggested much lower risk based on statistical corrections to exclude various biasing factors [15]. Retrospective studies on the natural history of HNPCC have shown that the colorectal cancer risk begins already around the age of 20 and has a peak incidence between the ages of 40–50 years [16, 17]. Therefore surveillance colonoscopies should start soon after the age of 20, and at age 25 years at the latest. There is no real upper limit for continuing surveillance but the potential benefits decrease after the age of 80 years suggesting discontinuing repeated examinations at that age at the latest [18]. Naturally, the general condition of the subjects under surveillance must be taken into consideration.

Endoscopic surveillance decreases colorectal cancer incidence on the basis that adenomatous polyps are removed as a part of the examination. In addition, incident cancers are also predominantly detected in an asymptomatic phase and therefore, show a more favorable stage distribution compared to historical non-screened control patients from the same HNPCC families [19, 20]. The most conclusive evidence in favor of surveillance comes from a prospective family cohort study of 22 HNPCC families with 252 healthy at-risk persons followed up for 15 years [21]. Colonoscopy was offered for all, but 133 subjects participated in surveillance with 3-year intervals while 119 others either declined or were not reached. The study ended when molecular testing of the mutation status became possible and most subjects accepted testing. At the end of follow-up, colorectal cancer incidence was reduced by 62% in the screening group, and colorectal cancer deaths were completely prevented compared with nine deaths among 19 patients with colorectal cancer in the control group, Table 18.4. The effect was due to polypectomies in altogether 31 cases of the surveilled subjects compared to none in controls until genetic testing and endoscopy was finally arranged for all mutation-positive subjects. It is worth noting that the colorectal cancer burden in HNPCC is so heavy that there was a difference of overall mortality in favor of the surveillance group.

Table 18.4 Tumor stage distribution in a 15-year follow-up study of 252 at risk members of 22 HNPCC families [21]

Tumor stage (Dukes)[a]	Surveillance group ($n=133$)	Control group ($n=119$)
A	3	3
B	5	7
C	–	1
D	–	8
Benign adenoma	31	4[b]
Colorectal cancer in all	8[c]	19
Colorectal cancer death	–	9
All neoplasms	39	23

[a] Tumor stages were significantly more favorable in the surveilled group ($p=0.03$)

[b] Detected in surveillance colonoscopy outside the plan [2] or after disclosure of a mutation-positive test [2]

[c] The number of colorectal cancer cases was significantly less in the surveilled group than in the controls ($p=0.014$; relative risk 0.377)

The appropriate time interval between surveillance visits remains under debate in the lack of comparative studies. The 3-year interval used in the 15-year trial described above more than halved the cancer incidence and prevented cancer deaths fully. Actually, the reduction of cancer incidence was even higher if the two cancer cases detected at the first examination were excluded as they were not begun at the optimal starting age of 25 years or earlier. On the other hand, more frequent endoscopy, for example, by 2-year intervals would probably further reduce the colorectal cancer incidence if more adenomas could be detected and removed. In a Dutch series of 666 mutation carriers from 110 families, 41 colorectal cancers were diagnosed during endoscopic surveillance [18]. Colorectal cancers occurred after different interval lengths, but most tumors were still local (stage A or B) and only five of 34 cancers (15%) were of stage C with metastatic regional lymph nodes. The authors concluded that the use of a 2-year interval between examinations would have made an earlier cancer diagnosis possible in more than half of these cases, but that a yearly endoscopy would not have added the effectiveness.

The quality of the bowel preparation and of the examination itself are at least as important as the length of the interval, because advanced cancer very probably means a missed early lesion in the previous examination. Further accuracy for the detection of minor lesions may be achieved by using dye spraying or magnifying endoscope. On the other hand, a certain miss rate of minor polyps must be accepted as a risk of the process. Virtual colonoscopy or computed tomographic colon examination has been recently introduced as an alternative to colonoscopy even though it does not enable biopsy or treatment. In HNPCC, the detection rate of small polyps was found all too inaccurate for routine use even though large lesions were detected [22]. At present, computed tomographic colonography can only be used as a complementary tool under special circumstances. In repeated examinations, excessive cumulative radiation exposure becomes problematic, but the situation may change when magnetic resonance colonography becomes available.

Because the inconvenience, costs, and even risks of the repeated colonoscopies are not negligible, the optimal interval should be examined in a randomized trial. Considering the ages from 25 to 80 years, the total number of colonoscopies will count 55, 27, or 18 depending whether yearly, biannual or triennial examinations are used. Very importantly, the surveillance program should be tolerable for the subjects so that the compliance would be as complete as possible also in long term. Sufficient pain relief is one important aspect of the examination having influence both on the detection rate of small lesions and especially on the compliance.

18.4.2 Surveillance for Gynecological Cancer

Endometrial cancer is the second most common cancer associated with HNPCC with a cumulative incidence between 40 and 60% in lifetime which may actually exceed the risk of colorectal cancer in women [23]. In addition, the risk of endometrial cancer seems even higher in mutation carriers of the MSH6 gene [23]. Therefore, gynecological examination including transvaginal ultrasonography and aspiration biopsy has been recommended by 2–3-year intervals for women starting at age from 30 to 35 years. Transvaginal ultrasound examination also enables visualization of the ovaries with an estimated lifetime cancer risk of 12% [3]. The advisability of surveillance has, however, been questioned because the outcome of patients with endometrial cancer is relatively favorable in general; the 5-year survival in endometrial cancer lies around 90%. On the other hand, screening for ovarian cancer, with much poorer outcome, has thus far been disappointing in general, and the accuracy of ultrasound to differentiate suspicious changes from benign lesions of the ovaries is poor.

The effectiveness of transvaginal ultrasound has been examined in two trials. In a British–Dutch study, 269 women at 50% risk of having HNPCC had 522 transvaginal ultrasound examinations by 1–2-year intervals without finding cancer or premalignant lesions [24]. Two women, however, presented with symptoms 6 and 24 months after a normal ultrasound examination and were diagnosed to have early endometrial cancer (FIGO I). In another Dutch study, 41 women from Lynch syndrome families (11 mutation-positive subjects) had yearly ultrasonography with the finding of thickened endometrium in some cases. This led to the diagnosis of complex atypical hyperplasia in three cases, a premalignant lesion [25]. A third trial included 175 proven mutation carriers followed up for median 3.7 years using transvaginal ultrasound added with endometrial aspiration biopsy by 2–3-year intervals. Endometrial cancer occurred in 14 cases, 11 of which were diagnosed by surveillance, one in prophylactic hysterectomy specimen, and only two presented by symptoms in the interval. In addition, there were 14 other cases with various stages of premalignant hyperplasia, including complex atypical hyperplasia in five cases [26].

It appears that the use of endometrial aspiration biopsy in addition to transvaginal ultrasonography significantly increases the efficacy of gynecological surveillance and may justify gynecological surveillance at least in mutation-positive subjects.

There is no definite evidence about the most appropriate surveillance interval, but probably an interval of 2–3 years as used in colon examinations is sufficient. However, there has been no support to the early detection of ovarian cancer by surveillance.

18.4.3 Surveillance for Other Related Cancers

For many other cancer types involved with mismatch repair gene mutations, such as gastric, uroepithelial, kidney, small bowel or biliary tract cancers, and brain tumors there are no reliable or easy methods for early detection. Furthermore, in most of these tumor types, the cumulative risk remains so small that any meaningful surveillance program could be cost-beneficial. The risk values have been estimated to less than 10% with the exception of gastric cancer with a risk of 13% at the age of 70 years [3]. The use of upper gastrointestinal endoscopy was examined in a group of 70 mismatch-repair gene carriers in comparison with their mutation-negative siblings without finding any cases of gastric cancer or more premalignant lesions in mutation carriers [27]. Surveillance gastroscopy has sometimes been advised in those HNPCC families where more than one case of gastric cancer has been observed. This idea suggests that there may exist some mutation types or modifying genetic factors, which increase the occurrence of gastric cancer. Another modifying factor may lie in the environment causing increased gastric cancer risk in the general population. Thus, surveillance of the stomach might be worthwhile in high-risk regions, such as China, Japan, or Korea. It may also be possible that occurrence of Helicobacter pylori or atrophic gastritis in the context of mismatch-repair gene mutations forms a significant additional risk indicating surveillance. At the present stage of knowledge, there is little justification for routine upper gastrointestinal endoscopic surveillance in HNPCC.

Surveillance of uroepithelial cancer has been suggested as an option to consider. The Danish HNPCC registry followed this recommendation using routinely urine cytology analysis for altogether 990 persons at risk who gave 1,868 urine samples [28]. The number of abnormal findings was 47 (2.5%), of which 31 were false positive, and 15 did not lead to further study (ignored). There was only one true positive finding leading to the detection of transitiocellular tumor (0.05%). It was concluded that screening for urinary tract tumors by using urine cytology is not justified.

Surveillance has not been examined in other tumor types with a probable association with mismatch-repair dysfunction, and no clear recommendations have been made. In this connection, it must be remembered that adding many diverse screening tools in the surveillance program without clear documentation of benefit may only unnecessarily increase the anxiety and decrease the compliance in the beneficial part of the surveillance. Awareness of the possibility of several tumor types should, however, lead to appropriate examinations in the case that atypical symptoms occur.

18.5 Alternative Strategies

18.5.1 Prophylactic Surgery

In familial adenomatous polyposis, colectomy with ileorectal anastomosis or total proctocolectomy with ileoanal anastomosis is the treatment of choice. The risk of colorectal cancer in familial adenomatosis is only slightly higher than in mutation carriers of mismatch-repair genes. The difference lies in the number of colonic adenomas, which are very numerous, from hundreds to thousands in familial adenomatosis but few in HNPCC. Another difference seems to be in the time table of adenoma progression to cancer, which is relatively slow in adenomatosis but rapid in HNPCC. The situation in the two conditions is similar enough to make prophylactic colectomy an attractive option also in mutation-positive subjects of HNPCC families. In addition to the substantial reduction of the colorectal cancer risk the examination of the residual rectum and sigmoid colon (after ileosigmoidal anastomosis) is much more easy and reliable while the functional harm due to increased bowel frequency remains minimal. Still, undertaking abdominal colectomy in HNPCC would mean an unnecessary operation with all its risk in some 20% or more of mutation-positive subjects. Cancer risk is lower in women who would probably have less benefit from prophylactic surgery of the colon. There may also be difference in the risk figures depending on the specific mismatch-repair gene and mutation in question.

There are, however, certain situations where prophylactic surgery is clearly recommendable, such as the finding of multiple adenomas difficult to remove, suspicion of early invasive carcinoma within a lesion with severe dysplasia, or repeatedly found adenomas in successive examinations. Also technically difficult or painful examinations may be resolved with prophylactic surgery [29]. In practice, very few mutation-positive subjects have chosen prophylactic colectomy in the absence of any tumor in their colon. In the Finnish HNPCC registry, the proportion of such subjects is less than 1%. It should be stressed that continuing surveillance of the remaining rectum and sigmoid colon is still necessary after colectomy.

A recent retrospective study reviewed a group of 315 mutation-positive women from Lynch syndrome families, 61 of whom had had prophylactic hysterectomy and ovarectomy [30]. There were no cases of endometrial or ovarian cancer among these subjects whereas one-third of women with no prophylactic surgery developed endometrial cancer and 5.5% of them ovarian cancer. Thus, prophylactic surgery is a very powerful tool in preventing gynecological cancer, and considering ovarian cancer, possibly the only way. It should be offered as an option when family planning has been completed, probably around the age of 40 years. In the Finnish surveillance study of 175 mutation-positive women, 43 women (25%) opted for prophylactic hysterectomy, including 11 cases diagnosed with premalignant hyperplastic change in aspiration biopsy [26]. However, in the case of endometrial cancer, regular aspiration biopsy and transvaginal ultrasound seems a reasonable choice.

18.5.2 Chemoprevention

A further preventive tool may come available from chemoprevention using nonsteroidal anti-inflammatory drugs, such as aspirin, sulindac or the more specific cyclo-oxygenase-2 inhibiting agents, the COX-2 blockers shown to reduce the number and size of sporadic adenomas or those occurring in familial adenomatous polyposis [31, 32]. A large international collaborative study organized in England is designed to test the effect of aspirin in Lynch syndrome patients, and results are awaited within few coming years. Whether or not aspirin or other chemoprevention drugs could reduce the risk in addition to surveillance alone will be determined based on the results from ongoing studies. The possibilities to reduce the risk of endometrial cancer by hormonal manipulation are also being studied[33]. In future, chemoprevention may offer additional reduction of cancer risk in HNPCC but can hardly replace surveillance.

18.6 Summary

Lynch syndrome causes an increased susceptibility for many cancer types, but colorectal cancer and endometrial cancer are by far the most important of these with lifetime incidences from 40 to 80%. Endoscopic surveillance can reduce the incidence of colorectal cancer to less than half and prevent colorectal cancer deaths. The effect is due to polypectomies and, at least, detection of early cancers in an asymptomatic stage. The colorectal cancer burden of subjects with Lynch syndrome is so high that surveillance improves the overall survival of the mutation-positive population. Endometrial cancer surveillance is also recommended with the use of endometrial aspiration biopsy combined with transvaginal ultrasonography. Final evidence of the efficacy of gynecological cancers is still lacking but it enables the detection and treatment of early endometrial cancer and premalignant hyperplasia. Prophylactic surgery, colectomy and/or hysterectomy with ovarectomy remain alternative options for cancer prevention in selected cases. Chemoprevention using aspirin or other nonsteroidal anti-inflammatory drugs for colorectal cancer or hormonal therapy for endometrial cancer may offer further risk reduction in future. In other associated cancer types with risk figures around 10% or less, such as cancers of the stomach, urinary tract, bile ducts or small bowel, and of brain tumors, there are no efficient tools for cancer prevention available.

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Part VI
Clinical Science: MYH Associated Polyposis

Chapter 19
MUTYH-Associated Polyposis

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Abstract *MUTYH*-associated polyposis is an autosomal recessive syndrome predisposing to colorectal cancer. The syndrome was described in the last decade and is characterized by an inherited deficiency in base excision repair. The natural history of the syndrome is still being described. This chapter will address the advances made in *MUTYH*-associated polyposis.

Keywords MAP • *MUTYH* • Base excision repair

19.1 *MUTYH*-Associated Polyposis

MUTYH-Associated Polyposis (MAP) is an autosomal recessive trait of adenomatous polyposis of the colorectum that carries a very high risk of colorectal cancer (CRC) [1–4]. MAP-associated tumours are characterised by acquired G to T transversion mutations. These appear to reflect an inherited deficiency in base excision repair (BER) that results directly from biallelic germ line mutations in *MUTYH*. MAP was first described in 2002 and clinical studies are just beginning

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to define the phenotype and natural history of the disorder, while genetic, cellular, and transgenic approaches are probing the mechanisms that lead to its development. In contrast to other genes associated with inherited CRC syndromes, no evidence has been found to date to support a role for somatic inactivation of *MUTYH* in sporadic CRC.

19.2 *MUTYH* Mutations in *MUTYH*-Associated Polyposis

To date, the LOVD MUTYH database [5] contains 167 different likely pathogenic mutations of *MUTYH*, most of which have been reported in a variety of biallelic combinations in patients with MAP. MAP-associated mutations are distributed along the length of the gene, with the exception of the extreme 3′ region (exon 1). Splicing mutations have been reported in introns 1, 4–6, 8, 10, 11, 12, and 15. Missense changes account for 107/167 (64%) of mutations identified, nonsense mutations for 11%; 9% are predicted to affect splicing, 12% are small insertions or deletions, and <5% are in-frame insertions or deletions. The *MUTYH* reference sequence annotation has been modified since its initial characterization (GenBank accession: U63329.1) and the most up-to-date annotation (NM_001128425.1) is used in this chapter to describe mutations. The changes induced occur after nucleotide c.157 (exon 3, codon 53) and result in the extension of numbering by 42 nucleotides (14 amino acids) compared to the previously used sequence.

As of 2008, 766 mutant *MUTYH* alleles had been reported in the literature in apparently unrelated MAP index cases [3, 4, 6–47]. We have recently undertaken a European collaborative study of MAP in Germany, The Netherlands, and UK which identified over 200 further mutant alleles in apparently unrelated index cases [48, 49]. Y179C and G396D (previously known as Y165C and G382D) are ten and seven times more prevalent in reported MAP cases than any other mutation (Fig. 19.1) and predominantly occur in the Western population. Together they account for 70% of reported mutant alleles, but there has also been a reporting bias towards these two mutations as mutation-specific assays have been undertaken to identify MAP cases in many studies. In addition, mutation frequencies vary between populations, for example, Y179C and G396D have not been reported in Japanese MAP patients [24, 50] and mutations c.1147delC (previously c.1105delC) and c.1437_1439delGGA (previously c.1395_1397delGGA) appear to be more frequent in patients of Mediterranean origin [12, 13]. As more studies are reported that employ full sequencing of the *MUTYH* open reading frame (ORF) and as further populations are studied, a more accurate picture of the distribution of MAP-associated mutations will be obtained.

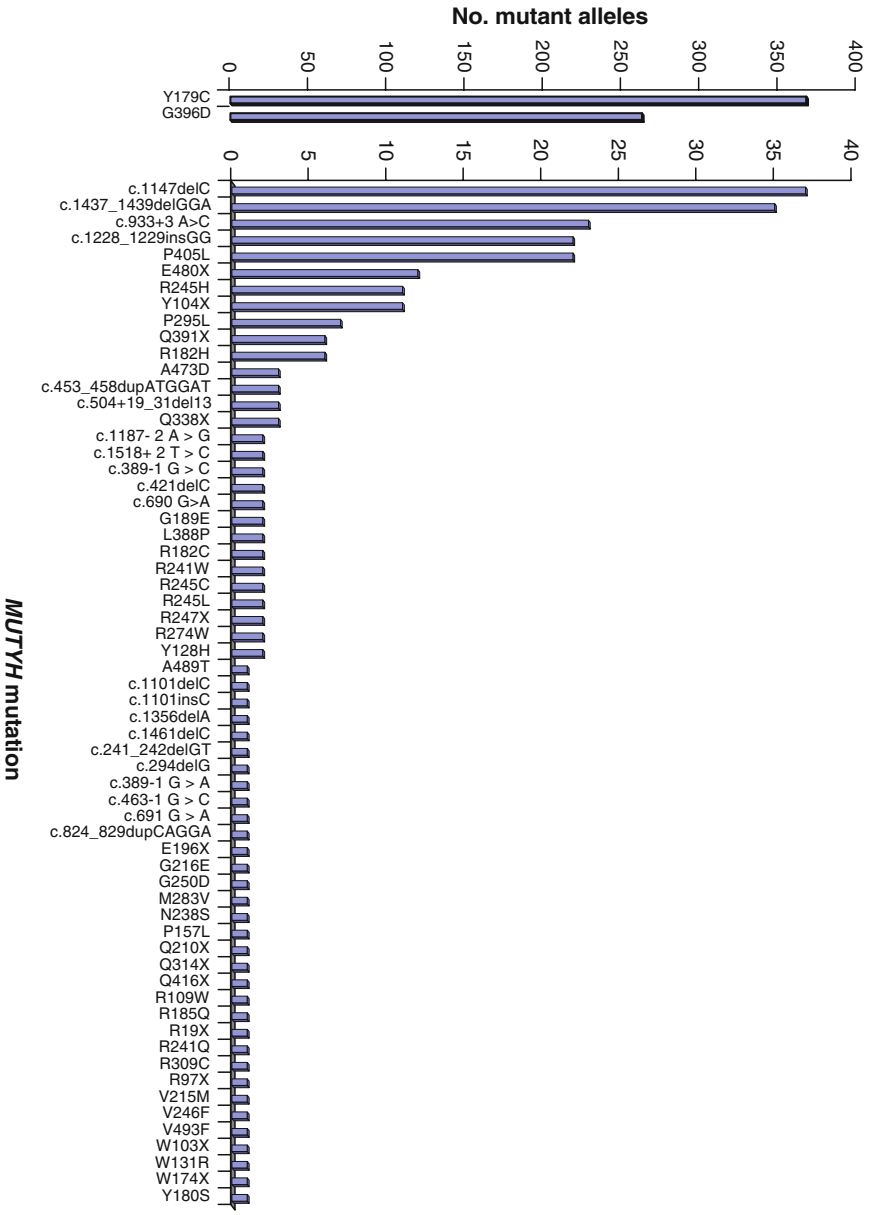

Fig. 19.1 Frequency of *MUTYH* mutations previously reported up to 2008 or identified in MAP cases in the European collaborative study. Only mutations identified in unrelated MAP patients with biallelic *MUTYH* mutations are shown [3, 4, 6–49]

19.3 Colorectal Phenotype

Several small studies of the MAP colorectal phenotype have now been described [3, 6, 16, 17, 25, 29]. These suggest that development of tens to hundreds of colorectal adenomas before the fifth or sixth decade is typical. A small number of individuals with biallelic mutations appear to still be polyp-free in mid-life [10, 17], while exceptionally over a thousand adenomas have been reported [13, 33]. We have undertaken a European collaborative study of MAP in Germany, The Netherlands, and UK which sought to improve the characterization of the MAP phenotype and has collated data on 257 MAP patients, of whom 185 were index cases and 72 were affected relatives [49]. Of these, 108 were female (42%) and 149 (58%) were male. The mean age at presentation of the 172 MAP patients who presented symptomatically was 45 years (median 45 years, range 12–70 years), consistent with previous smaller series [6, 12, 17, 18, 23, 43]. Only a single patient presented symptomatically under the age of 20 years (with more than a hundred colorectal polyps but no CRC) and just 5% of the cases presented before the age of 30 years. Only five patients first came to medical attention at over 65 years of age; one without a known cause of presentation, three with symptoms, and one through a population screening programme. All but one (who presented symptomatically) had CRC at presentation. These extremes were, therefore, very rare and the majority of patients (70%) who presented symptomatically came to medical attention between the ages of 35 and 54 years.

The colorectal phenotypes of MAP patients in our European collaborative study closely resembled that of attenuated familial adenomatous polyposis (AFAP, with typically <100 adenomas) or were more similar to that of classical familial adenomatous polyposis (FAP, with typically 100–1,000 adenomas). Of the cases, 52% had 10–100 adenomas, 25% had 100–1,000 adenomas, 7% had less than ten adenomas, and 15% were recorded only as having "polyposis" or "multiple polyps." Although we did not identify any patient who had developed CRC in the absence of coexisting polyps, some previous studies have reported such patients [10, 17, 42] and according to a large population-based CRC cohort, up to one-third of biallelic *MUTYH* mutation carriers develop CRC in the absence of multiple adenomas [51]. It is likely that these phenotypic differences reflect differences in ascertainment, since most cases in our European collaborative study were identified through polyposis registers, while studies that have identified biallelic mutation carriers with CRC but without apparent polyposis have screened cohorts of CRC patients. This suggests that current screening strategies may fail to identify a significant number of MAP patients. Two MAP cases with over a thousand colorectal polyps have been reported [13, 33], but none of the patients in our European collaborative study had such severe polyposis.

In most MAP cases identified by our European collaboration, all characterised polyps were adenomas but in less than 10% of the patients, a small number of hyperplastic polyps were identified in addition to adenomas. Colonic hyperplastic polyps and serrated adenomas are increasingly reported in MAP patients [3, 27, 31,

44, 46, 52, 53] and there is evidence that such lesions may arise due to MUTYH deficiency [53]. A link between hyperplastic polyposis syndrome (HPS) and CRC through a serrated adenoma pathway has been proposed [54], but it is unknown whether CRC in MAP can arise from hyperplastic and adenomatous polyps.

Of the MAP patients in our European collaborative study, 58% (148/254) had been diagnosed with CRC. Previous studies that reported more than ten MAP patients identified CRC in 29% [23], 44% [17], 50% [3], 62% [13], and 75% [12] of cases, but all these studies were very small (21 patients or less). In the European collaboration, CRC affected 82/149 (55%) males with MAP and 66/108 (61%) females; 78% (116/148) had CRC at presentation and 20% (30) developed CRC later (two patients with unknown age at first diagnosis). The mean age at first CRC diagnosis was 48 years [49], which is consistent with previous reports [12, 17, 38, 43, 44]. A quarter of CRCs were diagnosed between 45 and 49 years of age but the age at first CRC diagnosis ranged from 21 to 70 years (Fig. 19.2). Therefore, the European collaborative study concluded that the distribution of age at onset of CRC in MAP is several years later than that in classical FAP but earlier than that in AFAP [55]. The mean age at CRC diagnosis in Lynch syndrome of 45 years [56] is similar to that in MAP but the lifetime CRC risk may be higher in untreated MAP patients, particularly in females [22, 57], as suggested by a recent large population-based series which estimated a CRC penetrance of biallelic mutations of approximately 70% at 70 years of age [58]. Adenoma initiation in MAP is probably neither as frequent nor early as in classical FAP since, as in AFAP, biallelic somatic mutations of APC appear to be required (rather than just a single somatic mutation as in

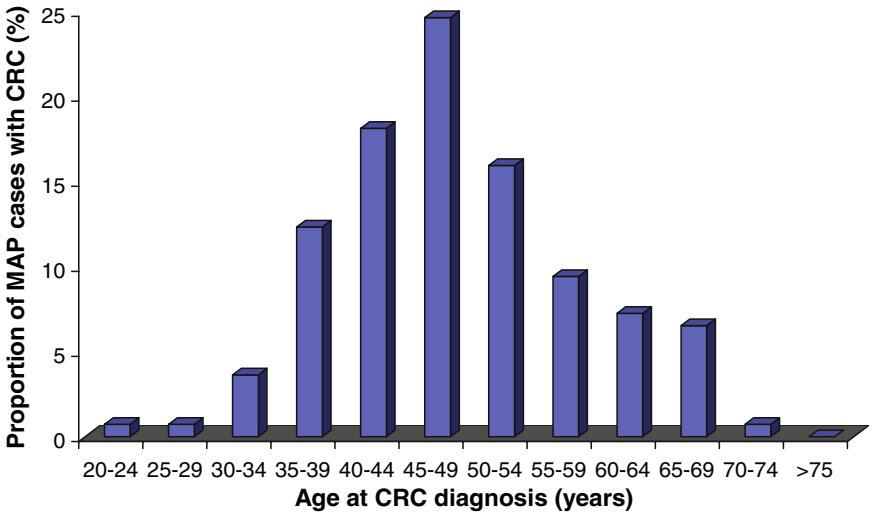

Fig. 19.2 Age at CRC diagnosis of 148 MAP patients with CRC. A quarter of MAP cases with CRC were diagnosed between the ages of 45 and 49 years. Only 5% of cases were diagnosed before the age of 35 years. The age at diagnosis ranged from 21 to 70 years [49]

classical FAP). However, the BER defect in MAP may result in an increased risk of progression and earlier onset of CRC than in AFAP.

Of the MAP patients diagnosed with CRC in our European collaborative study, 33% (49/148) developed at least one synchronous or metachronous CRC. Several previous reports have also described MAP cases with more than one CRC [6, 12, 16, 34, 44, 59]. The risk of synchronous or metachronous CRC may, therefore, be significantly higher in MAP patients than that in Lynch syndrome patients [56, 60].

Of the 138 MAP patients with CRC in the European collaborative study for which tumour location data were available, 44% had left-sided CRC (i.e. at or distal to the splenic flexure) and 56% had right-sided CRC. This is in contrast to the much smaller series (24 cases) reported by Lipton et al. [59] who observed predominantly left-sided CRCs in MAP patients. Left-sided CRCs are thought to be more likely to behave aggressively than proximal (right-sided) CRCs [61]. Classical FAP CRCs show a left-sided predominance [55, 62], whereas more AFAP and Lynch syndrome CRCs are right sided [57, 62, 63]. In MAP patients in the European collaborative study, 24% (48/200) of CRCs of known location were located in the rectum or rectosigmoid. This is a lower proportion than previously reported in MAP patients [6, 33, 34, 44, 59]. Rectal cancer is also observed in FAP patients [62] but is rarely seen in AFAP [64] or Lynch syndrome cases [63].

19.4 Extracolonic Manifestations

Although a variety of extracolonic pathologies have been reported in MAP patients, many have not been described at significant frequencies, making their true association with MAP unclear [6, 13, 42, 65–67]. The European collaborative study examined retrospective data on extracolonic manifestations in 276 MAP cases (Table 19.1) [48]. Duodenal and gastric polyps are the most frequently reported extracolonic findings [3, 6, 33, 35, 43, 65] and were identified in 17% and 11% of patients screened, respectively. Gastric carcinoma was identified in three of 276 MAP patients in the European collaborative cohort, but the incidence was not higher than that in the general population (Table 19.1) and this tumour has been reported previously in only a single case [65]. Jejunal polyps have been identified in two MAP patients [35, 48]. Despite small intestine cancer being rare in the general population, accounting for 0.4% of new cancer cases in USA in 2005 [68], two duodenal carcinomas have been previously reported [65, 66] and a further two cases were identified in our study (Table 19.1). As for FAP and Lynch syndrome [55, 60], we found a high relative risk for this cancer in MAP patients (SIR: 129; 95% CI: 16–466) and the cumulative lifetime risk was estimated at 4% [48]. Carcinoid tumours have not been reported before in MAP cases and are rare in the general population [69], but we identified two MAP patients with a small intestine carcinoid tumour and two others had a carcinoid tumour of the appendix. Larger studies are required to clarify the likely aetiological association.

Table 19.1 Frequency of extracolonic cancers observed more than once in 276 MAP patients in the European collaborative study [48]

Site of cancer	Sex	*n*	SIR (95% CI)	Obs %-risk by 75 years (95% CI)	Median age at diagnosis in years (range)	Ref. previous report
All extra-intestinal malignancies[a]	Both	44	1.9 (1.4–2.5)	38 (23–52)	54 (27–78)	
Oesophagus	Both	2	5.5 (0.7–19.8)	2 (0–4)	53 (46, 59)	–
Stomach	Both	3	4.2 (0.9–12.3)	1 (0–3)	38 (17–48)	[65]
Duodenum	Both	2	129 (15.7–465.9)	4 (0–9)	61 (56–65)	[65, 66]
Bladder	Both	4	7.2 (2.0–18.4)	6 (0–12)	61 (45–67)	–
Skin[b]	Both	13	2.8 (1.5–4.8)	17 (4–29)	58 (30–71)	[67]
Lung	Both	2	0.6 (0.1–2.3)	3 (0–8)	60 (51, 69)	–
Breast	F[c]	8	2.1 (0.9–4.2)	25 (0–51)	53 (45–76)	[6, 13, 42, 65]
		11[d]	3.0 (1.5–5.3)		55 (45–78)	
	M	1	53.5 (1.4–298)	1.5 (0–4.5)	56	–
Ovary[c]	F	3[e]	5.7 (1.2–16.7)	10 (0–22)	51 (45, 56)	–
Endometrium[c]	F	2	4.6 (0.6–16.5)	3 (0–7)	51 (47, 54)	[67]

The incidence of extra-intestinal malignancies in MAP patients in the European collaborative cohort was nearly twice that of the general population, and the lifetime risk approached 40%, although no predominant tumour and no shift towards early onset manifestation was observed [48]. We observed a wide range of tumours (Table 19.1), which points to a certain phenotypic overlap with Lynch syndrome. Patients affected by the Lynch syndrome variant Muir Torre syndrome develop sebaceous adenomas and carcinomas [56]. Two MAP patients have been reported previously to have sebaceous adenomas [27, 66] and a single case to have sebaceous carcinoma [67]. Our European collaborative study identified a further four MAP cases with sebaceous adenomas and a single case with sebaceous epithelioma [48]. Such lesions are rare in the general population [70]. Four MAP cases had sebaceous gland hyperplasia but this is reported to occur in 1% of healthy individuals [46] and is generally considered to be of no clinical importance [70]. Ponti et al. [46] detected *BRAF* mutations in sebaceous hyperplasias from MAP cases but since the mutation involved was a T>A rather than a G>T change, the mechanism linking MAP to this skin pathology remains unclear. We also identified a single case that had been diagnosed with steatocystoma, a cystic lesion of the pilosebaceous unit.

Breast cancer has been reported previously in four MAP cases [6, 13, 42, 65] and was one of the most frequent extracolonic cancers seen in the European collaborative cohort of MAP patients. However, only 7% (8/118) of female MAP patients had been affected at a mean age of 57 years and this was not significantly different to observed incidence rates in the general population [69]. One of 158 males with MAP was affected by breast cancer at 56 years of age [48]. Despite a significant increase in breast cancer in the Dutch subgroup of our cohort [25], the ensemble of our data does not suggest a clinically significant increase in breast cancer risk in MAP.

FAP-associated extra-intestinal lesions were uncommon in MAP patients in the European collaboration, four cases had mandibular cysts and one had a benign bone tumour [48]. Osteomas, and dental and mandibular anomalies have been reported in other MAP patients [12, 23, 35], but the former two were absent in our cohort. Only three patients were diagnosed with CHRPE [48] and few cases have been reported previously [3, 6, 12, 34]. It appears that there is very limited extra-intestinal phenotypic overlap between MAP and FAP.

19.5 Genotype–Phenotype Relationship

The European collaborative study investigated the colorectal phenotypes of MAP patients carrying the most frequently observed mutant alleles, Y179C and G396D [49]. For this purpose, data on Y179C homozygotes, G396D homozygotes, and Y179C/G396D compound heterozygotes were compared (Table 19.2).

Table 19.2 Ages at presentation and CRC diagnosis of MAP cases in the European collaborative cohort according to genotype. Cases homozygous or compound heterozygous for the most frequently mutated alleles Y179C and G396D were analysed

Genotype	Mean age at presentation in years (range)	*n*	95% CI	Mean age at CRC diagnosis in years (range)	*n*	95% CI
Y176C/Y176C	43* (24–65)	42	40.1–45.4	46** (24–65)	42	43.8–48.5
Y176C/G393D	50* (12–68)	38	46.1–53.6	52** (30–67)	32	47.8–55.3
G393D/G393D	51* (36–62)	12	44.9–56.6	58** (37–70)	9	51.5–65.2

*$P = 0.001$ (linear regression); **$P < 0.001$ (linear regression) [49]

MAP patients with a homozygous G396D mutation or compound heterozygous Y179C/G396D mutations presented later ($P = 0.001$, linear regression) and had a significantly lower CRC hazard than patients with a homozygous Y179C mutation ($P < 0.001$, Cox regression analysis). The mean ages at CRC diagnosis were 58 years and 52 years for G396D homozygotes and Y179C/G396D compound heterozygotes, respectively, compared with 46 years for patients with a homozygous Y179C mutation ($P < 0.001$, linear regression) [49]. A smaller study had suggested previously that CRC risk might be higher in MAP patients with biallelic Y179C mutations than that in those with biallelic G396D mutations, but the differences were not statistically significant [71]. We found similar proportions of G396D homozygotes and Y179C homozygotes with synchronous or metachronous CRC (4/9; 44% vs. 18/42; 43%). No significant differences in CRC location or Modified Astler–Coller (MAC) stages were observed between the different genotype groups. However, we demonstrated that the number of Y179C alleles was inversely correlated with the proportion of cases with fewer than 10 colorectal polyps ($P = 0.006$, linear-by-linear association), with 20% of G396D homozygotes displaying this milder phenotype, whereas just 2% of Y179C homozygote patients had fewer than ten polyps.

The genotype–phenotype correlations observed in the European collaborative cohort are likely to reflect the different effects the mutations have on the MUTYH protein. Y179C has been found to have a more detrimental effect on MUTYH glycosylase activity than G396D, and Y179C homozygous cells have been reported to express low levels of mutant MUTYH protein, while G396D homozygous cells were found to express levels of MUTYH protein similar to that in their wild-type counterparts [72].

Although we have found evidence to support genotype–phenotype correlations in MAP, considerable variability in expression was seen between patients carrying the same *MUTYH* mutations, for example, one Y179C/G396D compound heterozygote aged 38 years had CRC and 100–1,000 polyps, while another aged 52 years had less than ten polyps and no cancer. Intrafamilial variation was also seen and has been previously reported [73]. Additional genetic and environmental factors must modify the MAP phenotype [74].

19.6 Heterozygous *MUTYH* Mutation Carriers

The most frequently observed MAP-associated mutant alleles, Y179C and G396D, are present as heterozygous changes in approximately 1–2% of the Western population [10, 22, 36, 39, 71] but in clinical practice, heterozygous mutation carriers are often identified during genetic testing of the families of MAP index cases. Despite a number of studies reporting an overrepresentation of *MUTYH* heterozygotes among CRC cases [10, 11, 22, 32, 36, 45, 51, 75] and independent statistical significance having been reached in three of these [22, 36, 51], the clinical significance of harbouring a single *MUTYH* mutation remains unclear.

Our European collaborative study determined the CRC incidence and mortality, all-cancer incidence and mortality, and all-cause mortality in 350 obligate *MUTYH* heterozygotes (all but three were the parents of unrelated MAP index cases) by comparing their retrospective data to appropriate national age-, sex-, and period-specific data for the general population [76].

A twofold increase in CRC incidence was detected among the obligate *MUTYH* heterozygotes (SIR 2.12; 95% CI: 1.30–3.28). As this is comparable with the relative risk of 2.24 observed in individuals from the general population with at least one first-degree relative affected by CRC [77], we suggest that screening measures for CRC in heterozygous relatives of MAP patients need to be no more intensive than for this group. Neither CRC mortality (SMR 1.02; 95% CI: 0.41–2.10) nor overall cancer risk (SMR 0.92; 95% CI: 0.70–1.18), cancer mortality (SMR 1.12; 95% CI: 0.83–1.48), or overall mortality (SMR 0.94; 95% CI: 0.80–1.08) was not significantly increased in *MUTYH* heterozygotes [76].

19.7 Genetic Testing and Clinical Management of *MUTYH*-Associated Polyposis

Although Y179C and G396D are the most frequent mutated *MUTYH* alleles reported in MAP patients, approximately 15% of cases in the European collaborative study carried neither of these common mutations. Some molecular genetic diagnostic services currently screen for these common mutations and undertake more comprehensive screening for *MUTYH* mutations only if one of these mutations is found. This approach will lack sensitivity. The wide spectrum of mutations across the gene that has already been identified in cases with MAP suggests that sequencing of the entire *MUTYH* ORF is justified in the diagnostic setting. This will inevitably identify variants of unknown significance, and the development of a robust functional analysis will be important in discriminating between pathogenic and non-pathogenic changes.

The findings of the European collaborative study support recent recommendations from a European expert group [68] that colonoscopic surveillance of patients with biallelic *MUTYH* mutations should begin late in the second decade as we

identified only a single MAP patient who presented symptomatically before the age of 20 years and none who developed CRC before this age. As there is some evidence that CRC can develop in patients with biallelic mutations late in life and in the absence of obvious polyposis [10, 22], surveillance should be life-long. In the European collaborative study, most CRCs were diagnosed at first presentation, including CRCs that occurred in siblings after the earlier presentation of the proband within the family. A more proactive approach including predictive genetic testing of siblings coupled to regular surveillance could reduce their associated morbidity and mortality. There is no data upon which to base decisions about the screening interval. Two-yearly screening was recommended by the European expert group [78] and is likely to represent an adequately cautious approach until more data are available. Further work is required to address the place of upper GI screening in MAP as duodenal and other small intestinal cancers appear to be an occasional manifestation. The European expert group suggested initiating upper GI surveillance at between 25 and 30 years of age [78]. Decisions on the nature and timing of colorectal surgery must be determined on an individual basis because the MAP colorectal phenotype is so variable and in some mildly affected patients, surgery might be delayed or avoided by intermittent colonoscopic polypectomy. Our retrospective study did not suggest a clinically significant increase in risk of cancers outside the gastrointestinal tract and we do not suggest any surveillance for extra-intestinal cancer, but large prospective studies are still required to define fully the natural history of MAP.

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Part VII
Clinical Science: Hamartomatous Polyposis

Chapter 20
Peutz–Jeghers Syndrome

Francis M. Giardiello

Abstract Peutz-Jeghers Syndrome is an autosomal dominant syndrome characterized by hamartomatous gastrointestinal polyps and mucocutaneous melanin pigmentation. Patients are also at risk for extraintestinal neoplasms. In this chapter, the clinicopathologic characteristics of the syndrome, its management and surveillance recommendations will be discussed.

Keywords Autosomal dominant • Hamartomatous polyps • Melanin pigmentation • Intussusception • Small bowel polyps

20.1 Introduction

Peutz–Jeghers syndrome (PJS) is an autosomal dominant disorder caused by germline mutation of the STK11/LKB1 gene and characterized by hamartomatous polyps in the gastrointestinal tract and mucocutaneous melanin pigmentation. PJS patients are at risk for small bowel intussusception in childhood and common and unusual types of gastrointestinal and nongastrointestinal tumors in adulthood.

The first literature report of PJS appeared in the case of J.T. Connor published in the Lancet in 1895 [1]. Dr. Connor described two identical twin sisters with labial and oral pigmentation. These twins were illustrated by British surgeon J. Hutchinson in the Archives of Surgery in 1896 and were known, forevermore, as the "Hutchinson twins" [2]. In 1921, Dr. Johannes Peutz, Chairman of Medicine at Westeinde Hospital in The Hague, Netherlands reported a Dutch family with pigmentation of the skin/mucous membranes and gastrointestinal polyposis. He also highlighted the autosomal dominant inheritance of the syndrome [3]. In 1949, Harold Jeghers, Chief of Medicine at Georgetown University, publishing in the New England Journal of Medicine [4] established the inherited condition of intestinal polyposis and pigmentation of the skin and

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mucous membranes as a distinct entity. In 1954, A. Bruwer coined the eponym "Peutz–Jeghers syndrome" in the title of his article [5] addressing this disorder.

Ironically, the yet to be appreciated sequeli of PJS were exemplified in the medical history of the two Hutchinson twins. One twin died from intestinal obstruction at age 20 and the other from breast cancer at age 59 [6].

20.2 Clinical Manifestations

PJS is an inherited disorder characterized by mucocutaneous pigmentation and hamartomatous polyps in the gastrointestinal tract. The diagnosis of PJS can be made in patients with Peutz–Jeghers polyp(s) with at least two of the following clinical criteria also present: labial melanin deposits, a family history of the syndrome, or small bowel polyps [7]. The syndrome is found in all racial groups and has equally sex distribution. The incidence of PJS ranges from estimates of 1/50,000 [8] to 1/200,000 live births [9].

20.2.1 Clinical Presentation

The presenting complaints of PJS include intestinal obstruction (43% of the time), abdominal pain (23%), hematochezia (14%), and anal extrusion of polyp (7%). The remaining patients (13%) come to medical attention because of investigation of melanin pigmentation of the lips or bucal mucosa. The most frequent complication in young age is intussusception of the small bowel, which occurs in 47% of patients. Most affected persons become symptomatic between the ages of 10 and 30 [8]. The average age of diagnosis of PJS is 23 in males and 26 in females.

On physical examination the *sine qua non* of PJS is mucocutaneous pigmentation which occurs in infancy and can fade in late adolescence [10] (Fig. 20.1). These pigmented macules are dark brown, 1–5 mm in size, and located on the vermillion border of the lips (94% of patients), bucal mucosa (66%), hands (74%), and feet (62%). Periorbital, perianal and genital pigmentation has also been described. These pigmented spots are caused by pigment-laden macrophages in the dermis and are present in more than 95% of affected patients [8]. In contrast, freckles are not located on the buccal mucosa or copiously around the mouth and nostrils. Similar type and location of pigmentation can be seen in other conditions including the Laugier–Hunziker syndrome [11] and isolated melanotic mucocutaneous pigmentation (IMMP). Patients with IMMP have labial pigmentation histologically similar to PJS, no small bowel polyps or mutation of the SKK11/LKB1 gene, but females may have an increased risk of breast and gynecologic cancers [12].

20.2.2 Gastrointestinal Polyps

In one literature report, gastrointestinal (GI) polyps were noted in 88% of patients with PJS [8]. PJS polyps are found primarily in the small intestine but commonly

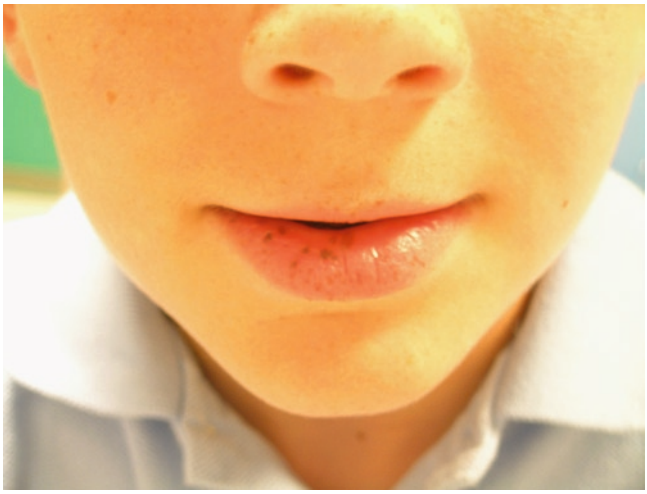

Fig. 20.1 The labial melanin pigmented macules noted in Peutz–Jeghers syndrome

in the colon and stomach. Polyps are seen at the following locations and frequency: small intestine (64%), colon (64%), stomach (49%), and rectum (32%) [8]. They number between one and 20 per GI segment and have variable size (0.1–5 cm in diameter) [13]. In case reports, PJS polyps (with the appropriate epithelium for the area) can be found in the renal pelvis, urinary bladder, lungs, and nares [14–16].

The Peutz–Jeghers polyp has unique histopathologic features and meets the definition of hamartoma (Fig. 20.2). These characteristics include frond-like structure, appropriate epithelium for each area of the gastrointestinal tract, and associated smooth muscle proliferation. Microscopically, the Peutz–Jeghers polyp consists of an arborizing framework of connective tissue and smooth muscle lined by normal intestinal epithelium, with abundant Goblet cells with long, elongated, and convoluted glands.

20.2.3 Gastrointestinal and Nongastrointestinal Neoplasia

Utsunomiya et al. in 1975 described the natural history of PJS in a cohort of Japanese patients [8]. The survival rate of PJS patients was 60% at age 60 compared to the 85% for the general Japanese population but better than the 5% survival rate of persons with familial adenomatous polyposis. Seeking an explanation for the decrement in survival in PJS adults, in 1987 Giardiello et al. first reported a strikingly increased risk of gastrointestinal and nongastrointestinal cancer in PJS [7]. These investigators reported gastrointestinal and nongastrointestinal cancers in 15 of 31 (48%) patients with PJS calculating the relative risk of cancer in these individuals at 18 times the general population risk. Consequently, this concept was supported by several other literature reports [17, 18].

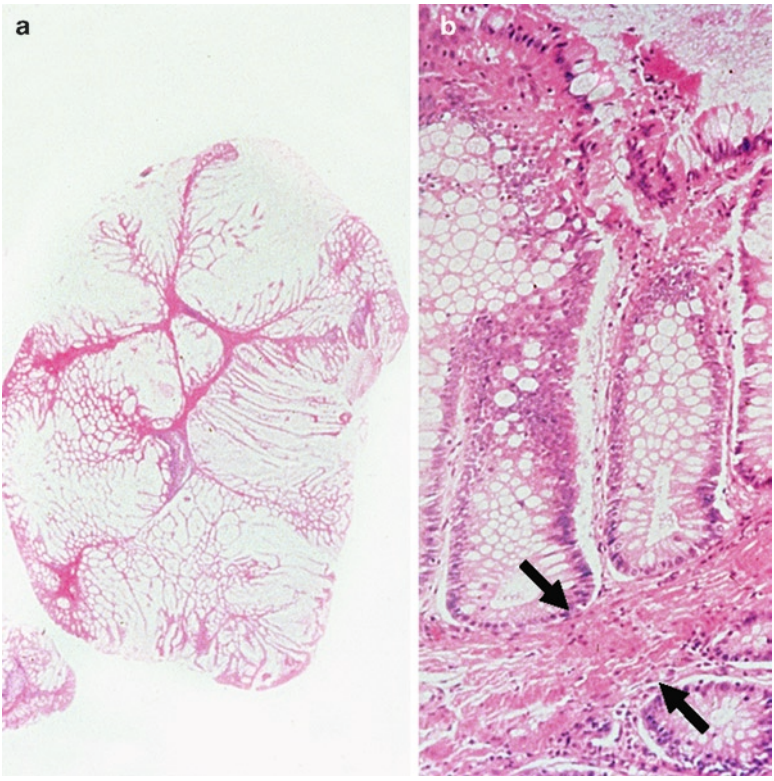

Fig. 20.2 **(a)** Low power view of Peutz–Jeghers polyp with branching framework of connective tissue. **(b)** High power view of smooth muscle (between arrows), lined by normal intestinal epithelium

In 2000, a meta-analysis of cancer risk in 210 PJS patients diagnosed by clinical criteria described in six publications revealed a relative risk for all cancers of 15.2 (95% confidence limits [CL], 2.0–19.0) and a lifetime risk of any cancer of 93% [19]. The gastrointestinal cancers at increased risk included esophagus, stomach, small intestine, colon, and pancreas (Table 20.1). In addition, a high risk of nongastrointestinal cancers including lung, breast, uterus, and ovaries (Table 20.1) was noted. Analysis of this data was sobering for several reasons. First, virtually all PJS patients were predicted to develop one or more common malignancies during a lifetime. Second, the absolute risk for breast cancer in PJS was similar to the magnitude noted in hereditary breast cancer caused by germline mutations of BRCA1 and BRCA2. Third, PJS was as strong as any risk factor for pancreatic cancer conferring a 36% lifetime risk of this malignancy in PJS patients. Fourth, cancer occurs at young age (Table 20.1).

Additional evaluation of cancer risk in PJS was reported by Lim W et al. [20]. This investigation evaluated 240 individuals with known mutation of the STK11 gene and found the risk of cancer by age 70 was 81%. The most common cancers identified were gastrointestinal in origin (esophagus, stomach, small bowel, colorectum,

Table 20.1 Cumulative risk of cancer by age 64 and mean age of diagnosis and range of age of diagnosis of cancer[a]

Site	Cumulative risk to age 64	Mean age of diagnosis	Range of age of diagnosis
All cancers	93%		
Esophagus	0.5%		
Stomach	29%	30	16–44
Small intestine	13%	42	32–51
Colon	39%	46	32–59
Pancreas	36%	41	24–58
Lung	15%		
Testes[b]	9%	9	4–13
Breast	54%	37	28–46
Uterus	9%		
Ovary	21%	28	24–32
Cervix	10%	34	23–54

[a]Cancer risk from meta-analysis and mean age of diagnosis of cancer and range age of diagnosis of cancer from literature case reports from ref. [19]
[b]All were sertoli cell tumors

pancreas). The cumulative risk for these cancers at age 60 was 42%. The risk of breast cancer in women was substantially increased, at 32% by age 60.

PJS is also associated with unusual types of tumors including, in females, sex-cord tumor with annual tubules of the ovary and adenoma malignum of the cervix [21]. Adenoma malignum is a very well-differentiated cervical cancer, so well differentiated toward normal epithelium that this lesion can escape detection on pap smear. In males, testicular tumors of sex cord and Sertoli-cell type have been associated with sexual precocity and gynecomastia in boys with this syndrome [22].

20.3 Genetic Cause

PJS is inherited as an autosomal dominant disorder with incomplete penetrance and variable expression. In 1998, two different European laboratories determined the cause of PJS as mutation in the STK11 (serine threonine kinase 11) gene, also known as the LKB1 gene found on chromosome 19p13.3 [23, 24]. The STK11/LKB1 gene is 23 kb long, composed of 9 exons, encoding a 433 amino acid serine threonine kinase protein [23, 24] and thought to be a tumor suppressor gene [25]. Most mutations in PJS patients include nonsense deletions, insertions, and rearrangements which lead to null alleles [26]. Of the known STK11 mutations, about 65% affect the protein structure.

The serine/threonine kinase acts as a regulator of cell-cycle metabolism. In addition, this gene belongs to a family of genes, which directs the execution of a cellular polarity program. Disruption of these genes in various cell and murine model systems results in epithelial and mucosal prolapse a defect postulated by some investigators as the cause for the formation of the Peutz–Jeghers polyp [27].

Approximately 30–70% of sporadic cases of PJS and 70% of affected individuals with a family history of the condition are found to carry a STK11/LKB1 gene mutation. The rate of *de novo* mutation of this gene presenting as spontaneous cases of PJS is unknown. Inability to identify a STK 11/LKB1 gene mutation in all affected patients suggests either limitation of current molecular techniques, genetic mosacism, or additional PJS loci [28–30]. With regard to the former, exonic STK11/LKB1 deletions are a common cause of PJS with rates varying from 14% to 16% [31, 32]. These genetic abnormalities are not readily detectable by standard sequencing technology. With regard to the latter, some studies suggest linkage to loci on chromosome 19q and 16q [33, 34].

Few genotype–phenotype studies have been done in PJS. One study noted that individuals with missense mutations had a significantly later time of onset to first polypectomy and other symptoms compared with individuals with truncating mutations or no detectable mutations [30]. Another investigation reported that the risk of intussusception in PJS is not influenced by STK11 mutation status [35]. The risk of cancer does not appear to be different in patients with and without identified mutations [36].

20.4 Management

20.4.1 Screening of At-Risk Individuals

Experts recommend screening at-risk individuals (first degree relatives of PJS patients) starting at birth with annual history and physical examination with evaluation for melanotic spots on the lips, buccal mucosa, above the eyelashes, and on the digits. Also examination for precocious puberty, and testicular tumors is recommended [37].
Genetic testing for STK11/LKB1 mutation should be offered to at-risk individuals who are asymptomatic and without stigmata at age 8 [38, 39]. This young age of genetic screening is recommended to make a presymptomatic diagnosis of PJS and plan elective prophylactic surgery if necessary avoiding the higher morbidity of emergent laparotomy for small bowel obstruction (secondary to small bowel intussusception caused by large polyps – occurring in about 30% of PJS patients by age 10) [40, 41].

Genetic testing is performed by *first testing* an affected member of the family with PJS to identify the pedigree mutation. Once the pedigree mutation is found (which can be done in up to 70% of affected individuals) at-risk individual can have site-specific mutation testing producing definitive true-positive or true-negative test results. At-risk members with true-negative test results have a risk of PJS similar to that of the general population. At-risk relatives who test positive have PJS and should follow the surveillance guidelines as described below.

If a STK11/LKB1 gene mutation is not identified in an affected family member, testing at-risk relatives is inappropriate because the gene test will be inconclusive. Consequently, at-risk members are advised to pursue regular small intestinal contrast radiography every two years until 25 years old [40]. Other authorities suggest upper endoscopy, colonoscopy, and small bowel series at ages 12, 18, and 24 [41]. Patients with melanotic pigmentation but uninformative genetic testing should follow surveillance guidelines.

Presently, clinical genetic testing for mutations of the STK11/LKB1 gene is available through several commercial laboratories. The primary method utilized is sequencing of the entire coding area. Some laboratories also perform deletion and duplication analysis, which appears necessary to identity about 15% of mutations. The price of testing ranges from $975 to $1,400 for identification of the proband mutation with significant reduction of cost for evaluation of at-risk family members for a known pedigree mutation. For additional information on commercial genetic testing, go to http://www.genetests.org.

20.4.2 Surveillance of Affected Individuals

As noted above, individuals affected with PJS are at-risk for a wide variety of gastrointestinal and nongastrointestinal cancers and other tumors at young age. No controlled studies on the effectiveness of cancer surveillance in PJS exist. However, recommendations derived from expert opinion are espoused in the literature. In a recent publication [42], the risks of specific tumors in PJS are analyzed in concert with published expert opinion regarding cancer monitoring and surveillance recommendations. Table 20.2 lists the surveillance recommendations by organ. Table 20.3 arranges these recommendations by sex and age.

Table 20.2 Surveillance recommendations by organ

Cancer type	Age at initiation	Surveillance
Breast[a]	18	Breast self exam – monthly
	25	Clinical breast exam – semiannually
	25 (or earlier based on earliest age of onset in family)	Mammography – annually (MRI offered as alternative)
Colon	18	Colonoscopy – every 2–3 years
Pancreas	25–30	Endoscopic ultrasound – every 1–2 years (CT scan and/or CA-19-9 offered as options)
Stomach and small intestine	8	Baseline upper endoscopy and small bowel series
	18	Upper endoscopy and small bowel series (Capsule endoscopy alternative) – every 2–3 years
Ovaries	25	Transvaginal ultrasound and serum CA-125 – annually
Uterus and cervix	21	Pelvic exam with pap smear – annually
	25	Transvaginal ultrasound and serum CA-125 – annually
Testicles	Birth	History and physical exam with attention to examination of testicles and routine blood tests – annually (Ultrasound of the testicles every 2 years until age 12 offered as an option)

[a] Discuss option of prophylactic mastectomy on a case-by-case basis and counsel regarding degree of protection and reconstruction options. The benefit of chemoprevention is unclear

Table 20.3 Surveillance recommendations by sex and age

Age (years)	Males	Females
Birth to 12 years	History and physical exam with examination of testicles and routine blood tests – annually (Ultrasound of the testicles every 2 years until age 12 offered as an option)	History and physical exam with routine blood tests – annually
At age 8	Upper endoscopy, and small bowel series – If positive continue every 2–3 years	Upper endoscopy, and small bowel series – if positive continue every 2–3 years
From age 18 on	Colonoscopy, upper endoscopy, and small bowel series (Capsule endoscopy alternative) – every 2–3 years	Colonoscopy, upper endoscopy, and small bowel series (Capsule endoscopy alternative) – every 2–3 years
		Breast self exam-monthly
From age 21 on	–	Pelvic exam with pap smear – annually
From age 25 on[a]	Endoscopic ultrasound – every 1–2 years (CT scan and/or CA-19-9 offered as options)	Endoscopic ultrasound – every 1–2 years (CT scan and/or CA-19-9 offered as options)
		Clinical breast exam – semiannually Mammography – annually (MRI offered as alternative)
		Transvaginal ultrasound and serum CA-125 – annually

[a] Mammography may begin earlier based on earliest age of onset in family

20.4.3 Treatment

Most experts recommend polypectomy for polyps in the stomach or colorectum measuring greater than 1 cm in size noted during endoscopic surveillance [13, 39].

Surgery is suggested for symptomatic or rapidly growing small intestinal polyps or asymptomatic polyps over 1–1.5 cm in size [9, 13, 38–40, 43]. Some authorities suggest attempting to clear the small intestine of polyps "clean sweep" during laparotomy. This is often accomplished by concomitant interoperative endoscopy with polypectomy or surgical enterotomy. The clean sweep approach appears to decrease the need for recurrent small bowel surgery [44]. Recently, the use of double balloon enteroscopy for removal of small bowel PJS polyps has been reported and, in skilled hands, appears to be a viable alternative to laparotomy [45].

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Chapter 21
Clinical Aspects of Juvenile Polyposis

Daniel Calva and James R. Howe

Abstract Juvenile polyposis (JP) is a hamartomatous polyposis syndrome, characterized by autosomal dominant transmission. Patients with JP have a propensity to develop polyps throughout the large bowel, and some will also develop these in the upper GI tract. The polyps can be numerous or few, and are not adenomas, as seen in familial adenomatous polyposis. The disease can be familial or sporadic (non-familial), and there is an increased risk for cancer of the GI tract. In this chapter, we will review the history, clinical features, histopathology, risk of malignancy, therapeutic options, and genetics of JP.

Keywords Hereditary • Mixed • Polyposis syndrome • Multiple • Adenoma • Patients

21.1 Historical Perspective

In 1914, Hertz described four children with rectal polyps in one family, where he felt there was a familial tendency. If in fact these cases were JP, this would be the first recorded case of Familial JP [1]. However, the first likely reported case of JP dates back to 1939, when Diamond described a 30-month-old child with a congenital polyp that prolapsed on defecation. The symptoms of the child were primarily constipation, with bright red blood per rectum, and on proctoscopy both

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a pedunculated and a sessile polyp were noted. The polyps described would now be recognized as hamartomatous polyps, with cystic glands that were distended, branching, and filled with fibrinous material. The polyps were covered by columnar epithelial cells, with inflammatory cells infiltrating the polyp [2].

In 1946, Helwig reported the histological findings of incidentally discovered polyps in patients less than 21-years-old from 449 consecutive autopsies. He described that the polyps in children were similar to those found in adults. He called these polyps adenomas without evidence of malignancy. However, histologically the polyps described were glandular structures filled with mucus, embedded in a stroma of cellular connective tissue, and infiltrated by inflammatory cells, as seen in juvenile polyps, without mention of adenomatous epithelium or dysplastic changes [3]. In 1948, Ravitch described a 10-month-old child that who on autopsy was found to have multiple polyps of the gastrointestinal tract, from the stomach to the anus. The child suffered from bloody diarrhea, and despite adequate nutritional support, he failed to gain weight. He was malnourished, cachectic, suffered from severe anemia requiring multiple blood transfusions, and had recurrent rectal prolapse and intussusception [4]. In 1951, LeFevre and Jacques also reported a 4-month-old child that died from ileocecal intussusception. The child presented with painless bleeding per rectum, anemia, and at autopsy he was found to have jejunal, ileal, and colonic polyps [5].

Horrilleno et al. performed a review of children with rectal and colonic polyps in 1957. Their contribution was in the histological analysis of the polyps, which were described as having proliferation of mucus-filled glands, retention cysts, abundant connective tissue, and a chronic cellular infiltration of eosinophils. They coined the term “hamartomatous polyp” based upon these observations [6]. In 1960, Knox et al. wrote a clinical and pathologic analysis of 43 patients with JP. They concluded that the hamartomatous polyp or juvenile polyp did not have malignant potential, nor was it a precancerous lesion [7]. It was not until 1962 that Morson distinguished the juvenile polyp from Peutz–Jeghers polyps, adenomatous polyps, and solitary polyps. He stated that juvenile polyps were malformations or hamartomas of the intestinal mucosa, without involvement of the muscularis mucosa layer, and without signs of hyperplasia, hyperchromatism, or increased mitotic activity [8].

In 1963, Roth and Helwig reviewed juvenile polyps from 158 patients. They reported a bimodal distribution to juvenile polyps, with 63% of patients presenting in childhood before the age of 10, and 37% in adulthood between the ages of 17 and 25-years-old. The oldest patient in the study was diagnosed with juvenile polyps at the age of 61. Ten percent of patients had auto-amputation of the polyps. They speculated that the bimodal distribution of the disease was due to the fact that the polyps appeared in childhood, but often underwent auto-amputation, leading to resolution of symptoms and findings, but recurred later in life [9].

McColl et al. was the first to coin the term “Juvenile Polyposis” in 1964, and felt that it was a different disease entity than adenomatous polyposis coli. He described two patients with severe anemia and hypoalbuminemia that underwent radical surgery, and noted that clinical symptoms usually began in childhood, with the average

age of onset at 6 years of age. Rectal bleeding was the most common symptom, followed by prolapse of the polyps per rectum. The polyps were described as bright red, smooth, sphere-like, and with a glistening surface [10]. In 1966, Veale et al. described the histopathology, clinical features, and family histories of 11 patients with JP. In the two families described, multiple family members had died from colonic, rectal or gastric cancer, but no link to cancer predisposition in JP was suggested. Histologically, the polyps were hamartomas without evidence of malignancy. The polyps varied from sessile to pedunculated, from a few millimeters to 3 cm in size, and the most striking feature of the polyps was the increased amount of supporting connective tissue compared to adenomas or other polyps. They concluded that the diagnosis of a juvenile polyp rested not only on the excess of connective tissue, but also on the lack of mitoses or hyperchromatism [11]. Smilow et al. described a 60-year-old diagnosed with an adenoma of the colon whose daughter and grandson had colonic polyps that histologically appeared to be hamartomas. They described one of the first examples of familial JP and the first recorded family with three affected members in three successive generations. They proposed a dominant pattern of inheritance and described the phenomenon of anticipation for the first time. Despite one individual with carcinoma, they felt that more evidence was needed to identify a malignant association [12].

In 1970, Sachatello et al. described a three generation family, with multiple of affected individuals with JP, whose stomach, small bowel, large bowel, and rectum were involved. This was the first study to describe generalized JP, that is, JP with both upper and lower GI polyps [13]. In 1971, Ray et al. reported a patient who was originally diagnosed with JP at 10 months of age. At 4 years of age, he had developed gastric and duodenal polyps as well, but had no family history of polyps or GI disease. Therefore, they described a case of sporadic generalized JP [14].

In 1975, Stemper et al. presented kindred with at least ten family members with single or multiple juvenile polyps of the stomach, small bowel, colon, and rectum. Ten members of the family had GI cancer and one had pancreatic cancer. They felt that the pattern of inheritance appeared to be autosomal dominant with high penetrance and pleomorphic phenotypes [15]. In 1978, Bussey et al. performed a comprehensive review of GI polyps and concluded that there are three types. These included hamartomatous polyp, which is seen in familial syndromes such as JP and Peutz–Jeghers syndrome (PJS), the inflammatory polyp, which is seen in conditions such as inflammatory bowel disease, and the neoplastic polyp. He noted that 20% of JP patients had some congenital anomalies, which include heart lesions, malrotation of the bowel, Meckel's diverticulum, and macrocephaly [16].

In 1979, Goodman et al. reported a 23-year-old female with rectal bleeding for 10 years who presented with epigastric pain. Multiple hamartomatous polyps were found in the stomach, colon, and rectum, which contained a spectrum of histological changes spanning from hyperplastic or juvenile to adenomas and adenocarcinomas. This was the first report indicating that juvenile polyps in JP patients may progress to malignant polyps [17]. That same year, Watanabe et al. reported a case of polyps of the stomach associated with gastric carcinoma, where the polyps were felt to be juvenile in nature [18].

21.2 The Current View

21.2.1 Definition

In 1974, Sachatello et al. were the first to define the criteria for the diagnosis of JP. The diagnosis is made by one or more of the following: (1) ten juvenile polyps in the colorectum; (2) juvenile polyps throughout the GI tract; (3) any number of juvenile polyps with a family history of JP [19]. In 1988, Jass et al. reviewed 1,032 polyps from 87 patients and proposed the number of polyps be reduced to five to meet the diagnostic criteria for JP when there is no family history [20]. In 1991, Giardiello was even less conservative, suggesting that all patients with three or more juvenile polyps are at risk for malignant transformation, and therefore should undergo regular screening [21].

21.2.2 Subtypes

In 1972, Sachatello et al. proposed the classification of JP into three subtypes: (1) JP coli, where the colon is the only site of polyposis (Fig. 21.1); (2) JP of infancy, which carries an unfavorable prognosis, since it has a higher incidence of larger recurrent polyps causing prolapse and intussusception; (3) generalized JP, where the distribution of the juvenile polyps occurs throughout the GI tract, although the

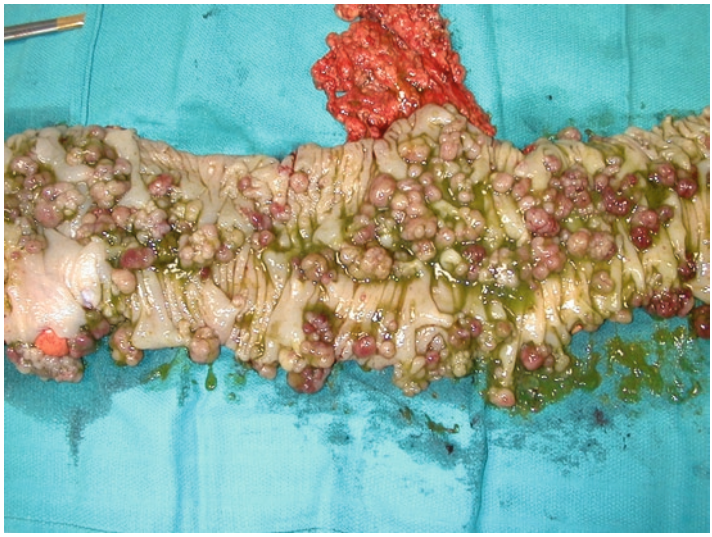

Fig. 21.1 Surgical specimen from a patient with JP with hundreds of juvenile polyps in the colon (from Merg et al. [84], by permission of *Current Problems in Surgery*)

Fig. 21.2 Surgical specimen of the stomach that is carpeted with juvenile polyps (from Merg et al. [84], by permission of *Current Problems in Surgery*)

site most commonly involved in the upper GI tract is the stomach (Fig. 21.2) [22]. This categorization continues to be used today.

21.2.3 Clinical Diagnosis, Workup, and Testing

In 1963, Roth and Helwig reviewed and reported on 158 patients with JP. They found that males were affected more than females, and Caucasians more than any other race [9]. However, Veale et al. reviewed 145 patients with JP, and did not find any difference in disease prevalence between males and females [11]. They did report that the average age of symptom onset was 6 years, with familial JP at 9.5 years, and sporadic JP at 4.5 years of age. The most common symptom was rectal bleeding, followed by prolapse of the polyps, mucus per rectum, diarrhea, and abdominal pain [11].

Grosfeld et al. described a family with JP in 1986, where he emphasized the differences between children with multiple juvenile polyps versus children with a solitary juvenile polyp. Juvenile polyps occur most commonly on the posterior wall of the bowel close to the blood supply. In contrast to patients with a solitary juvenile polyp, patients with multiple juvenile polyps may have considerable blood loss and present with iron deficiency anemia. In addition, due to the larger amount of mucus secretion from the polyps, these patients can present with significant protein loss leading to hypoalbuminemia, hypoproteinemia, anergy, and failure to thrive. Hypokalemia is also a result not only of the secreted mucus containing potassium,

but the massive diarrhea that develops. Other symptoms can include abdominal pain, clubbing, weakness, rectal prolapse, and intussusception [23].

Jass reviewed the pathology of various polyposis syndromes with special emphasis on JP in 1990. He reviewed the clinical feature of patients with a solitary juvenile polyps versus multiple juvenile polyps. Solitary juvenile polyps usually present in childhood, with the most common symptom being rectal bleeding, since the polyps tend to auto-amputate or prolapse per rectum. The polyps tend to mainly be located in the colon or rectum, and rarely in the small bowel. Children from ages 1–10 years are most often affected, with a peak incidence at age 4–5 years. The incidence of a solitary juvenile polyps is as high as 1% in children, who tend to have one or two, but rarely three or more polyps. Most cases of multiple juvenile polyps are sporadic, and account for 66% of cases, while 33% have familial tendencies along with an autosomal dominant pattern of inheritance [24]. If the condition is diagnosed in infancy, it is classified as JP of infancy, and this condition most commonly presents with severe diarrhea, protein losing enteropathy, prolapse, hemorrhage, malnutrition, intussusception, and electrolyte abnormalities. The entire GI tract is usually affected, and the prognosis is related to the extent of involvement, with the most severe cases leading to death before the age of 2 years [24, 25].

In 1995, Desai et al. published a review article on JP, where they found that JP patients have a variable number of polyps (between 50 and 200). There were 262 JP patients reviewed, and 98% of patients had polyps in the large bowel, which were evenly distributed throughout. In addition, 13.6% of JP patients were found to have gastric juvenile polyps, 2.3% had polyps in the duodenum, and 6.5% in the jejunum and ileum. In patients with generalized JP and JP coli, the most common presentation was bleeding per rectum, anemia, and prolapse of polyps. Eighty-five percent of these patients presented in the first or second decades of life, while 15% presenting in adulthood. Familial JP was found in 20–50% of patients, and all three subtypes are inherited in an autosomal dominant pattern [25].

Coburn et al. reviewed all the cases of JP published in the English literature up to the year 1995. There were 218 patients with a mean age at diagnosis of 18.5 years, which ranged from 9 months to 67 years of age. Fifty percent of patients had a family history of JP and 15% of patients had congenital anomalies. Anemia, rectal bleeding, prolapse of polyps, enteropathy, and intussusception were the most common presenting signs of JP [26].

21.3 The Juvenile Polyp

Helwig's study of 449 polyps from patients less than 21 years of age in 1946 led him to conclude that the polyps were adenomas without evidence of malignancy [3]. However, his description of polyps composed of glandular structures filled with mucin and embedded in a stroma of cellular connective tissue infiltrated by inflammatory cells is the hallmark of a hamartomatous polyp [3]. Horrilleno et al. coined the term "hamartomatous polyp" in 1957, noting that patients with JP had proliferation

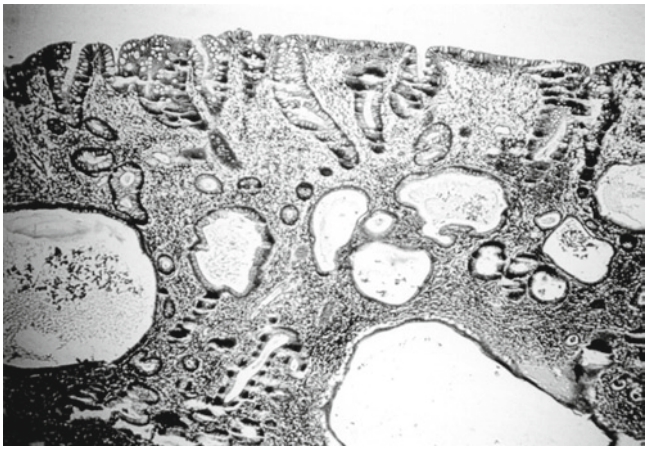

Fig. 21.3 The histological features of a juvenile polyp. There are cystic, glandular structures filled with mucus within the expanded lamina propria that are lined by epithelial cells. There is no muscularis mucosa in the lamina propria, but inflammatory cells can be seen infiltrating throughout (from Merg et al. [84], by permission of *Current Problems in Surgery*)

of mucus glands with formation of cystic structures in abundant connective tissue. There was also chronic cellular infiltration of eosinophils within the polyps (Fig. 21.3) [6]. In 1962, Morson felt that a juvenile polyp was a hamartoma, with malformation of the layers above the muscularis mucosa. The stroma of the polyp has tubules lined with columnar epithelium and many goblet cells, with atrophy of the lining epithelium in the tubules, showing cystic dilation and retention of mucus. In addition, typically there are no signs of hyperplasia, hyperchromatism, or increased mitotic activity. The epithelial element of the polyp is a continuous single layer of columnar and mucus-secreting cells, which covers the entire surface of the polyp. Some juvenile polyps might be complicated by ulceration, infection, or auto-infarction, and Morson felt that this might in part be an explanation for the infiltration of inflammatory cells [8]. Veale et al. provided a histologic description of polyps from 11 patients with familial JP in 1966. The polyps were sessile and pedunculated, and measured from a few millimeters to 3 cm in size (Fig. 21.4). The muscularis mucosa appeared normal and was not involved, and there were no signs of increased nuclear atypia, hyperchromatism, or mitosis. The polyps had more connective tissue compared to adenomas or other polyps [11].

In his 1990 review of JP, Jass concluded that the majority of polyps are typical; however, 20% of polyps are multilobated or papillary. The lamina propria may not be obvious and might be thinned out, and the epithelial cells lining the polyps may show dysplasia [24]. Solitary polyps generally have a smooth, spherical, red head, with a narrow stalk. The surface of solitary polyps has cysts filled with mucin, with no muscularis mucosa in the expanded layer, and the epithelium is normal and has no evidence of excess mitotic activity. There is also a strong component of infiltration of inflammatory cells in the lamina propria [24].

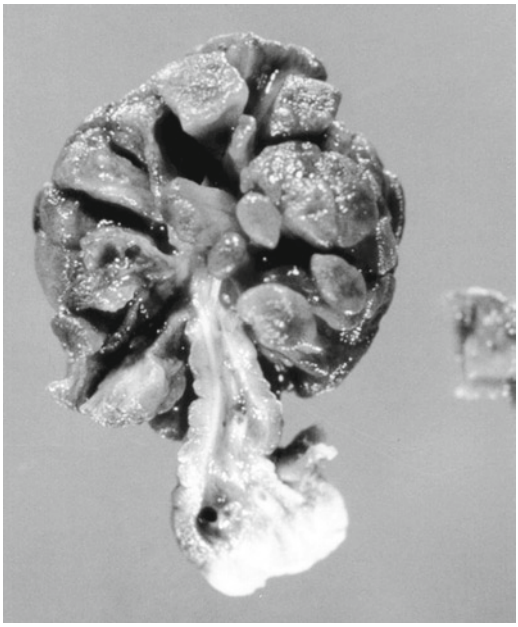

Fig. 21.4 A juvenile polyp, with a long stalk, removed at colonoscopy. Note the lobulated surface, which can become ulcerated and bleed

In 1993, Subramony et al. reported the findings from a large kindred with 41 individuals, in which 11 were diagnosed with JP. The study showed that in random biopsies of normal colonic mucosa, there was a dense population of mixed inflammatory cells infiltrating the upper half of the mucosa. Biopsies of nodular colonic/rectal mucosa showed cystic architectural changes with focal and diffuse inflammatory components raising the mucosa. They classified the polyps by size and found that polyps less than 1 cm showed typical histology to juvenile polyps; between 1 and 2.9 cm most polyps often had a pedunculated appearance with mildly dilated glands and epithelium showing mild to moderate dysplasia; and in polyps greater than 3 cm, the majority were pedunculated, with villi lined by mostly dysplastic epithelium resembling adenomas. The largest polyp had adenocarcinoma in the stalk of the polyp [27].

With respect to gastric polyps in JP patients, Subramony described them as having histological features identical to hyperplastic polyps, with the surrounding gastric mucosa showing a diffuse process consistent with chronic gastritis. In his report, one patient had gastric carcinoma, which was poorly differentiated and infiltrated the entire thickness of the stomach wall [27]. Sassatelli et al. published a case report of a 26-year-old male who was originally diagnosed with generalized JP at the age of 16. The polyps from the stomach showed a variety of histological features from hyperplastic or juvenile, to adenomatous polyps, and most had a mixed type. The most striking characteristic was that 5 years prior, at the age of 21, most polyps

were hyperplastic, and at the age of 26, most were of the adenomatous type, with one polyp having multiple foci of adenocarcinoma infiltrating the stroma. The largest polyp was 3.8 cm, and had hyperplastic, inflammatory, and adenomatous features. The adenomatous area showed foci of adenocarcinoma in situ. They proposed a sequence of events from focal mucosal hyperplasia to a hyperplastic polyp, which is indistinguishable from a juvenile polyp. With time, the polyps enlarge and develop changes within the epithelium to give it adenomatous features, with conversion to an adenoma, and ultimately carcinoma [28].

21.4 Genetics and Inheritance

The first observations that JP might be an inherited disorder date back to 1966 when Veale et al. described two families with multiple members affected by JP. In the first family, two sisters and their mother were diagnosed with JP; in the second family, two siblings were diagnosed with JP, but no one else in their family had JP or a history of colon polyps. However, their father was treated for inoperable rectal cancer and there was no mention of workup for polyps. Veale et al. suggested two hypotheses: one was that a "polyposis gene" produced juvenile polyps in the children, but in adults produced adenomas; the second was that environmental factors played a role in modifying the action of the "polyposis gene" to produce adenomas in adults and juvenile polyps in children [11]. The same year, Smilow et al. published a report of three patients in three generations in one family with JP and described anticipation, which is the phenomenon whereby the phenotype of a genetic disorder becomes apparent at an earlier age in subsequent generations, and sometimes with an increase in severity with each generation [12]. In 1975, Stemper et al. described a family where at least 15 members in two successive generations had JP. They felt that the inheritance was by a single autosomal dominant gene that had a high degree of penetrance and pleomorphic phenotypes. Their alternative hypothesis was that there were two closely linked autosomal genes, one coding for JP and the other for cancer [15].

In 1978, Bussey et al. performed a review of several gastrointestinal polyposis syndromes where a genetic factor seemed to play an important role. From the St. Mark's Hospital Polyposis Registry, 36 families were reviewed, with just over 50 JP cases. They concluded that in 25% of cases, there was a genetic defect that is inherited in an autosomal dominant pattern, since 25% of the families reviewed had several members affected in different generations. In the other 75% of JP patients, they believed a new genetic mutation or an environmental factor was more likely the culprit in these sporadic cases [16].

In 1993, Leggett et al. studied an Australian JP kindred with eight affected members in three successive generations. Six members of the kindred underwent colectomies in childhood, and two of them have been diagnosed with adenocarcinoma of the jejunum in adulthood. They excluded a gene for JP by linkage to chromosome 5, where the *APC* (adenomatous polyposis coli) and *MCC* (mutated in colorectal cancer) genes are located [29]. In 1997, Jacoby et al. described a 36-month-old neonate

with multiple associated extracolonic anomalies (clubfoot, broad nasal apex, long philtrum, widely spaced canthi, microcephaly, hypoplastic ears, umbilical hernia, tricuspid regurgitation, hypoplastic oblique muscles, short and broad feet and hands, and delay in motor and language skills), who was found on colonoscopy to have dozens of juvenile polyps throughout the colon, which recurred a year following polypectomy. Cytogenetic analysis showed a normal diploid chromosome number of 46, but an interstitial deletion was found on the long arm of chromosome 10 [30].

A major breakthrough in finding a gene responsible for JP was made in 1998 by Howe et al., who performed linkage analysis on affected members of the Iowa kindred, which was previously described by Stemper et al. They found linkage to chromosome 18q21, in a region encompassing the tumor suppressor genes *DCC* and *SMAD4*/*DPC4* [31]. Then they searched for germline mutations of both genes by sequencing and identified a 4-base pair (bp) deletion in exon 9 of *SMAD4* in all affected members of the kindred [31]. The findings of *SMAD4* germline mutations in JP patients was later confirmed by others [32–34]. *SMAD4* is the common intracellular mediator for the transforming growth factor beta, bone morphogenetic protein, and activin pathways, helping to transduce signals from cell surface receptors to the nucleus.

In 2001, Howe et al. examined four JP families without mutations of *MADH4* or *PTEN* (the gene for Cowden syndrome), by genetic linkage, and found linkage to chromosome 10. Mutations were found in all affected members of each family in the bone morphogenetic protein receptor type IA gene (*BMPR1A*), which maps to 10q22-23 near *PTEN* [35]. These findings were later confirmed in other JP families by Zhou et al. [36]. BMPR1A is a cell surface receptor of the TGF-β superfamily, which transduces signals from bone morphogenetic proteins into the nucleus through cytoplasmic SMAD4 and other co-SMAD proteins. In 2004, Howe et al. determined that germline mutations of *BMPR1A* and *SMAD4* together accounted for 40% of all the JP cases, and therefore there were likely other genes for JP not yet discovered [37].

21.5 Cancer Predisposition

21.5.1 Colorectal

When JP was initially described, the juvenile polyps were not considered to have malignant potential. In 1966, Veale et al. described two families where several members were diagnosed with JP. In the first family, two sisters and their mother had JP, and the maternal grandfather died of rectal cancer at the age of 57, and his daughter from a second marriage also died from colon cancer at the age of 32. In the second family, two siblings were diagnosed with JP, and their father had rectal cancer. They concluded that histologically the juvenile polyps do not have malignant features, and therefore there was not enough evidence to establish juvenile polyps as having malignant potential [11]. The progression of juvenile polyps to

cancer was not clear, and although several other families had also been described to infer this progression, the evidence was insufficient to clearly identify a causative relationship [12, 38].

In Stemper et al.'s 1975 report, the Iowa kindred consisted of 56 members, where 15 were diagnosed with JP and 11 had GI malignancies (five colon, two stomach, two duodenum, one pancreatic, and one unknown GI cancer). It was evident that cancer was common within three successive generations, and although they did suggest that the gene responsible for JP could possibly predispose individuals to the development of cancer, they failed to establish the malignant potential of JP since they had no evidence of carcinoma within juvenile polyps [15]. This evidence was provided by Tung-hua et al. in 1978, where they presented a case report of a 16-year-old who underwent polypectomy and histologically was found to have a typical juvenile polyp with foci of signet ring cell carcinoma [39].

Bussey et al. felt that the risk of adenomatous transformation in juvenile polyps was approximately the same for patients with a solitary juvenile polyp or JP [16]. Goodman et al. described a case report of a 23-year-old male with sporadic JP who was found to have rectal cancer. They described a spectrum of changes in the polyps (hyperplastic polyps or juvenile polyps, juvenile polyps with focal adenomatous changes, adenomas, and even adenocarcinoma) that led them to conclude that there is a pathogenetic sequence of events that leads the juvenile polyp to transform to adenocarcinoma [17]. Grigioni et al. also concluded that there is a pathogenetic sequence and proposed that the mucosa becomes hyperplastic, which leads to hyperplastic polyps; then the polyps become inflamed and enlarged secondary to ulceration and scarring. The crypts become dilated and full of mucus, assuming the appearance of a classic hamartomatous juvenile polyp. The polyp then gains some adenomatous features, which are followed by conversion to a tubular and/or villous adenoma, and finally adenocarcinoma [40]. Multiple other studies supported the findings that juvenile polyps have malignant potential in individuals with JP, however, the question of whether individuals with a solitary juvenile polyp were at risk for malignancy has still not been established [41–43].

Baptist et al. described a 17-year-old girl with two juvenile polyps of the colon and one >2 cm tubulovillous adenoma of the colon with carcinoma in situ [44]. Jones et al. published a case report of a 24-year-old patient with four colonic polyps in 1987. On pathologic examination an intramucosal carcinoma was found arising from a typical juvenile polyp. They concluded that although the juvenile polyps in individuals with JP have a particularly higher risk of malignancy, individuals with a solitary polyp should also be recognized as having malignant potential [45].

Jass et al. reviewed the clinical and pathological data of 87 patients with JP from the St. Mark's Hospital Registry in 1988. They analyzed 1,032 polyps, and found that 840 were typical juvenile polyps, 169 were multilobulated or villous, 21 were adenomas, and two were classified as hyperplastic polyps. They found that 9% of the typical juvenile polyps had dysplastic changes, and 47% of the villous polyps had dysplastic changes. Out of the 87 patients, 18 developed colorectal cancer, at a mean age of 34 years. They concluded that patients with JP and not solitary juvenile polyps

have a high risk of developing colorectal carcinoma [20]. Another case reported by Bentley et al. of a 38-year-old African–American woman with generalized JP added insight to the risk in patients with JP. In the stomach, all 16 polyps were typical juvenile polyps without adenoma or dysplasia. In the colon, 15 polyps were typical juvenile polyps, there was also a tubulovillous adenoma with infiltrating carcinoma, and another polyp had a focus of carcinoma in situ. They concluded that in patients with JP, the subgroup that has typical juvenile polyps with adenomatous features are at particularly high risk for the development of cancer [46].

Jass further clarified that the risk of malignant transformation is rare in solitary polyps, with only one case having been reported. In contrast, it was estimated that the cumulative risk of cancer in JP was likely in excess of 50% [24]. Another important prospective study published in 1991 by Giardiello et al. described four patients with JP, aged 3, 4, 6, and 7 years. Three of the four patients had no family history of JP or any colorectal disease. In two of the children, adenomatous epithelium was noticed in their polyps, and the other two children had colonic adenomas found. In the same study, they performed a retrospective review of 57 patients with JP, and analyzed all their polyps to evaluate the association with colorectal malignancy. Patients were classified as familial or sporadic, and as having JP or solitary juvenile polyps. Colorectal neoplasia was defined as having an adenoma, adenocarcinoma, or adenomatous epithelium within the juvenile polyps. The mean age of diagnosis of colonic neoplasia was 37 years for both sporadic and familial JP. They found that the risk of colonic neoplasia was about the same for both patients with familial JP (40%) or those with three or more juvenile polyps (47%). Therefore they concluded that patients with three or more juvenile polyps, and JP patients with or without a family history should undergo periodic screening with colonoscopy and polypectomy to screen for adenomatous changes and to remove polyps at high risk for malignant transformation. Furthermore, patients with a solitary juvenile polyp should also undergo polypectomy since there is a small but definitive risk for neoplasia, especially if there is a family history [21].

Coburn et al.'s 1994 review consisted of 218 patients, where GI carcinoma was found in 17% of the cases, with a mean age of diagnosis of 35.5 years. Seven patients with generalized JP had cancer, and 29 patients with JP coli had cancer. Eleven patients had rectal cancer, eight had cancer in the descending colon, four in the ascending colon, one in the duodenum, another patient had gastric cancer, and 11 patients had unspecified GI malignancies. In 31 patients, there was evidence of adenomatous changes in their polyps, and seven manifested foci of dysplasia. Malignancies were more common with JP coli, especially with the familial type. Men were affected more than females in this study, and more patients with JP coli versus generalized JP died from their malignancies [26]. It is important to mention that many, if not the majority of patients in the study, probably did not have upper GI evaluation to differentiate the diagnosis of generalized JP versus JP coli. Therefore, it is possible that many patients in the JP coli group could in fact have had generalized JP. It seems more likely that the generalized JP group carries a worse prognosis in terms of morbidity and mortality, as only patients having subtotal or

total gastrectomies were in this group, and overall, 30% of the generalized JP group had cancer as compared to 22% for the JP coli group.

Howe et al. reviewed the medical records and interviewed patients from the largest JP kindred described in the literature in 1998 (the Iowa kindred). The kindred had 117 members, and 29 of them had been diagnosed with juvenile polyps in the upper and/or lower GI tract. Eleven patients had developed colon cancer, four patients had stomach cancer, one patient had cancer of the duodenum/ampulla, and one patient had pancreatic cancer (Fig. 21.5). Therefore, in this family with generalized JP, the overall risk for colorectal cancer was 38%, upper GI cancer was 21%, and the overall risk for GI malignancies was 55% [47].

21.5.2 Gastric

There are several studies supporting the potential for malignant transformation of gastric polyps in JP patients. Stemper et al. was the first to describe gastric cancer in JP patients with gastric polyps, where two patients were reported [15]. In 1979, Watanabe et al. described two siblings with gastric juvenile polyps only, a 13-year-old girl and her 14-year-old brother. Their mother had passed away at the age of 37 from gastric cancer. Both patients underwent total gastrectomy, and their stomachs were carpeted with typical juvenile polyps, from the cardia to the pylorus, but with no evidence of dysplasia or atypia [18].

In 1988, Yoshida et al. described a 31-year-old male with generalized JP who was found to have a well-differentiated adenocarcinoma of the stomach. He was first diagnosed with colonic juvenile polyps when he was 17-years-old. The patient had a strong family history of gastrointestinal polyposis and malignancy, which included three individuals with gastric cancer, two with rectal cancer, and two with hepatocellular carcinoma. The polyps near the adenocarcinoma, but not on its immediate edge, were found to be typical juvenile polyps. However, polyps from the edge of the adenocarcinoma had structures resembling crypts lined by epithelial cells with dysplastic changes, and the surrounding mucosa near the ulceration had adenomatous changes. This study supported the notion that gastric polyps in patients with JP are at risk for malignant transformation, and that the disease is often a diffuse process in the stomach [48]. Sassatelli et al. described a similar phenomenon in 1993 in a 16-year-old male with generalized JP that was found to have diffuse polyposis of the stomach. At that time, most polyps were juvenile, but at the age of 21, most polyps were adenomas with one polyp along the greater curvature of the stomach having multiple foci of adenocarcinoma infiltrating the stroma [28].

In 1998, Howe et al. updated the findings from the Iowa kindred, earlier described by Stemper et al. [47]. They found that 6 out of 29 patients with JP had upper GI malignancies, and therefore the risk of developing upper GI cancer in this family with JP was approximately 21% [47]. Furthermore, patients that have *SMAD4* mutations have a more virulent form of JP, and have a higher risk of developing upper GI polyposis and cancer [49, 50].

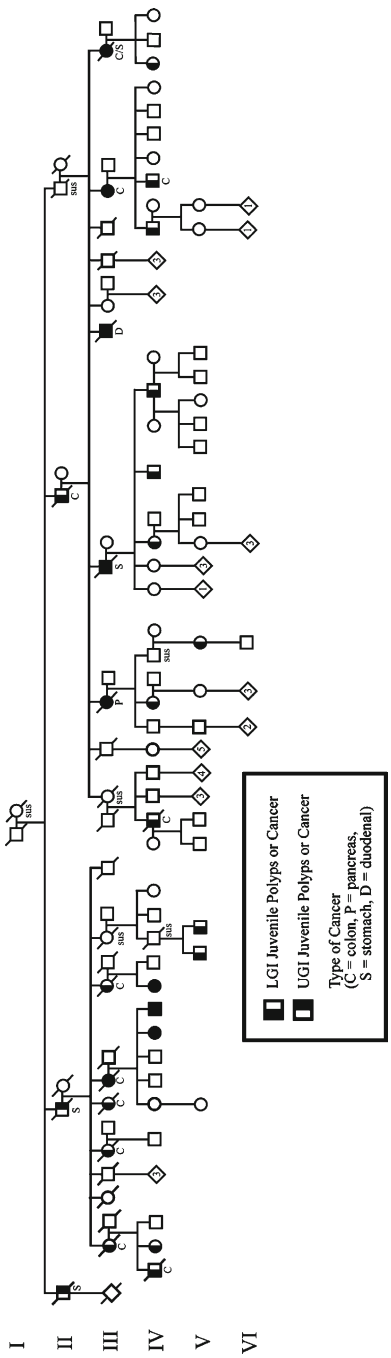

Fig. 21.5 The Iowa JP kindred, which is one of the largest in the literature (from Howe et al. [47], by permission of *Annals of Surgical Oncology*)

21.5.3 Pancreas

The first recorded case of pancreatic cancer in a family with JP was again described by Stemper et al. in the Iowa kindred in 1975 [15]. In 1989, Walpole and Cullity described a 19-year-old male with sporadic JP and adenocarcinoma of the pancreas. They concluded that there must be a genetic factor, which causes not only JP but also cancer in various tissues of the body, including the pancreas [51]. Howe et al.'s. 1998 update of the Iowa kindred still only recorded the one case of pancreatic cancer in 29 affected kindred members. In the accompanying review of the literature, the only other case of pancreatic cancer found in the literature was that discussed above by Walpole [47].

21.6 Differential Diagnosis

21.6.1 PTEN Hamartoma Tumor Syndromes: Cowden and Bannaya–Riley–Ruvalcaba Syndrome

These two syndromes share with JP that patients may develop hamartomatous polyps in the GI tract, but have other pathognomonic phenotypic characteristics that differentiate them from JP. Patients with Cowden syndrome often have benign or malignant lesions of the breast and thyroid gland. They also may have cobblestone-like papules of the gingiva and buccal mucosa, and multiple facial trichilemmomas [52, 53]. BRRS is distinguished from JP by characteristic macrocephaly, lipomatosis, angiomatosis, and pigmented macules of the body and glans penis [54–56]. Both Cowden and BRRS syndrome are caused by mutations in the tumor suppressor gene *PTEN*, which is located on chromosome 10 [57].

21.6.2 Peutz–Jeghers Syndrome

Patients with PJS also have hamartomatous polyps of the GI tract; however, jejunal polyps are a consistent feature, which is rarely seen in JP. More importantly, mucocutaneous pigmentation of the lips, buccal mucosa, and digits are pathognomonic in PJS [58, 59]. Histologically, the Peutz–Jeghers polyp, which is a type of hamartomatous polyp, is unique in that it has interdigitating smooth muscle bundles from the muscularis mucosa in a characteristic arborization, which is not seen in the juvenile polyp [60]. This syndrome is an autosomal dominant inherited disorder, where patients have germline mutations of the *STK11* gene on chromosome 19 [61, 62].

21.6.3 Hereditary Mixed Polyposis Syndrome

In Hereditary Mixed Polyposis Syndrome (HMPS), patients present at a median age of 40 years with signs and symptoms of bowel obstruction, altered bowel habits, rectal bleeding, abdominal pain, and laboratory evidence of anemia. Patients may have polyps throughout the entire colon, usually less than 15 at initial colonoscopy, and histologically these show evidence of a mixed picture from tubular adenomas, villous adenomas, and sessile adenomas to hyperplastic polyps and atypical juvenile polyps. Presently, it is not known whether HMPS is a distinct syndrome or not, but it seems to be inherited in an autosomal dominant fashion and carries a risk for the development of colorectal cancer [63].

21.6.4 Familial Adenomatous Polyposis

Although patients with JP were originally described as having adenomatous polyps in the colon, JP is characterized by hamartomatous polyps. JP is distinguished from FAP based on the type of polyps as well as in the clinical presentation. Individuals with FAP tend to develop hundreds to thousands of classic adenomatous polyps in the colon, usually beginning in their teenage years [64–66]. Typically, JP patients develop several to a dozen juvenile polyps, although some individuals will have up to 100 juvenile polyps in the colon. Other diagnostic features seen in FAP include congenital hypertrophy of the retina, jaw cysts, sebaceous cysts, osteomata, and desmoid tumors [67, 68]. FAP is a premalignant condition, where one or more polyps progress to malignancy if untreated, with a median age at diagnosis of 40 years. However, carcinoma may develop at any age, and patients may present with signs and symptoms of cancer, such as weight loss, bowel obstruction, or anemia from occult blood loss [64–66]. Mutations of the *APC* gene on chromosome 5 predispose to FAP [69, 70].

21.7 Associated Anomalies

Several authors have described associated extracolonic anomalies in JP patients, including macrocephaly, hypertelorism, amyotonia congenita, extra toes on the foot, Meckel's diverticulum with umbilical fistula, mild communicating hydrocephalus, malrotation of the gut, undescended testes, mesenteric lymphangioma, malrotation of the cecum, and acute porphyria [10, 11, 25]. In 1976, Raskin et al. reported a case of JP associated with Von Recklinghausen's disease [71]. In 1978, Bussey et al. added heart lesions to the list of anomalies that may be observed in JP, and estimated that 20% of patients with JP had congenital anomalies [16]. Desai et al. reported in their review that 11–20% of JP patients have extracolonic anomalies [25].

In 1989, Walpole and Cullity reported that a 19-year-old male JP patient with adenocarcinoma of the pancreas also had bilateral inguinal hernias in addition to macrocephaly, cryptorchidism, umbilical hernia, and clubbing of the fingers [51].

In Coburn et al.'s review, atrial septal defect was the most common thoracic cavity anomaly, but arteriovenous malformations (AVMs) of the lung, pulmonary stenosis, tetralogy of Fallot, coarctation of the aorta, patent ductus arteriosus and subvalvular aortic stenosis were also seen. Macrocephaly was the most common anomaly of the CNS, which also included hydrocephalus and spina bifida. Meckel's diverticulum was the most common of the GI tract anomalies, but gastric and duodenal diverticuli, and malrotation were also seen. Undescended testicle, unilateral renal agenesis, bifid uterus and vagina, and abnormal ureteropelvic insertion were the most frequent genitourinary anomalies. Osteoma, lymphangioma, pectus excavatum, hereditary telengactasia, familial congenital lymphedema, hypertelorism, thyroglossal duct cyst, and amyotonia congenita have also been described in JP patients [26]. Jass concluded that congenial anomalies were seen more commonly in sporadic than familial cases of JP [24]. In Desai et al.'s survey of the phenotypic features of JP, they found that 78% of all cases were males, and 22% were females [72]. However, five patients also had another genetic syndrome described (two BRRS, two had Gorlin syndrome, and one with Hereditary Hemorrhagic Telengactasia), raising some questions about the diagnosis [72]. It is possible that some of the extracolonic anomalies described in JP patients by Desai et al. and other authors could represent misdiagnoses, particularly those patients with macrocephaly who might have BRRS.

Cox et al. described a 28-year-old woman and her 10-year-old daughter with generalized JP, clubbing of the fingers, and AVMs of the pulmonary system in 1980. The daughter was diagnosed with juvenile polyps at the age of 5, and with AVMs at the age of 8 by pulmonary angiography. Her mother had an AVM of her lung resected at the age of 10, and a partial colectomy at the age of 12 [73]. In 1982, Conte et al. described a family where the father, his son, and daughter had JP, cutaneous telengiectasias, and pulmonary AVMs. The father died from colon cancer at the age of 36. They described the syndrome as an autosomal dominant disorder [74]. Baert et al. presented another case report in 1983 of a 15-year-old girl with juvenile polyps, clubbing of her fingers, and AVMs of the lung [75]. In 1999, Inoue et al. described a 14-year-old girl with generalized JP and telengiectasias of the skin, AVM of the right pulmonary artery, a dilated hepatic artery with intrahepatic AVM, and therefore was diagnosed with Hereditary Hemorrhagic Telengiectasia (HHT). She had no family history of polyps or vascular hereditary diseases, and her symptoms included rectal bleeding, anemia, and epixtasis [76].

Gallione et al. reported 14 patients (six families with a total of 13 individuals affected, and one individual without a family history) with JP and HHT in 2004. In this study, all 14 patients had mucocutaneous telengiectasias, seven had pulmonary AVMs, four had hepatic AVMs, one had a cerebellar cavernous hemangioma, nine patients suffered from epistaxis, and two had episodes of intracranial bleeding. Furthermore, they studied the genomic DNA of all six unrelated families and of the sporadic case and found that none of the patients had mutation in the *ENG* or *ALK1* genes, which have been associated with HHT, but instead, all had germline *SMAD4* mutations [77].

In 2006, Gallione et al. screened the *SMAD4* gene in 30 unrelated patients with HHT that did not have the *ENG* or *ALK1* mutations, and found three individuals who had mutations of *SMAD4*. All 30 patients in the study were not previously diagnosed with JP; however, on colonoscopy the three individuals with the *SMAD4* mutations were found to have colonic juvenile polyps, and one of the three had a history of colorectal cancer. Therefore, they proposed that individuals with HHT that did not have the *ALK1* or *ENG* mutations should be screened for the *SMAD4* mutation, and if the mutation is found, they would need to be screened for colonic and gastric polyps [78].

21.8 Management

21.8.1 Screening and Surveillance

Colonoscopy and EGD are the standard options for screening and evaluating the GI tract of JP patients. Capsule endoscopy, although less frequently used, has the potential to evaluate the entire GI tract, including a more thorough examination of the small bowel. For JP patients who have undergone surgical management, close screening and surveillance are required due to the high rate of recurrence of polyps. In patients at risk for JP, history and physical for evidence of signs or symptoms of JP (rectal bleeding, prolapse, anemia, constipation, obstruction, diarrhea, abdominal discomfort) should start at birth. EGD and colonoscopy should be done at the onset of signs and symptoms, or when the patient is 15 years old if they remain asymptomatic. If endoscopy is negative, then routine screening should be performed every 3 years. If polyps are found, they should be endoscopically removed if possible, and this should be repeated on a yearly basis until no polyps are found, at which time surveillance can resume in a 3-year interval. For individuals who do not have the mutation found in affected family members, if baseline screening is negative at 15 years of age, then screening should be repeated every 10 years until age 45; if no polyps are found, then screening should be done as for the normal population. If a genetic mutation is found in an at-risk patient, then screening every 1–3 years should be done, depending upon whether polyps are found. If no genetic mutation can be identified in the entire family, then screening is done as described for patients at risk for JP (Fig. 21.6) [47].

21.8.2 Operative Management for Colonic Disease

21.8.2.1 Polypectomy

In 1973, Sachatello et al. described the indications for operative management, prior to our understanding of the risk of malignancy in patients with JP. The options they discussed included: (1) polypectomy or fulguration of the polyps for rectal prolapse

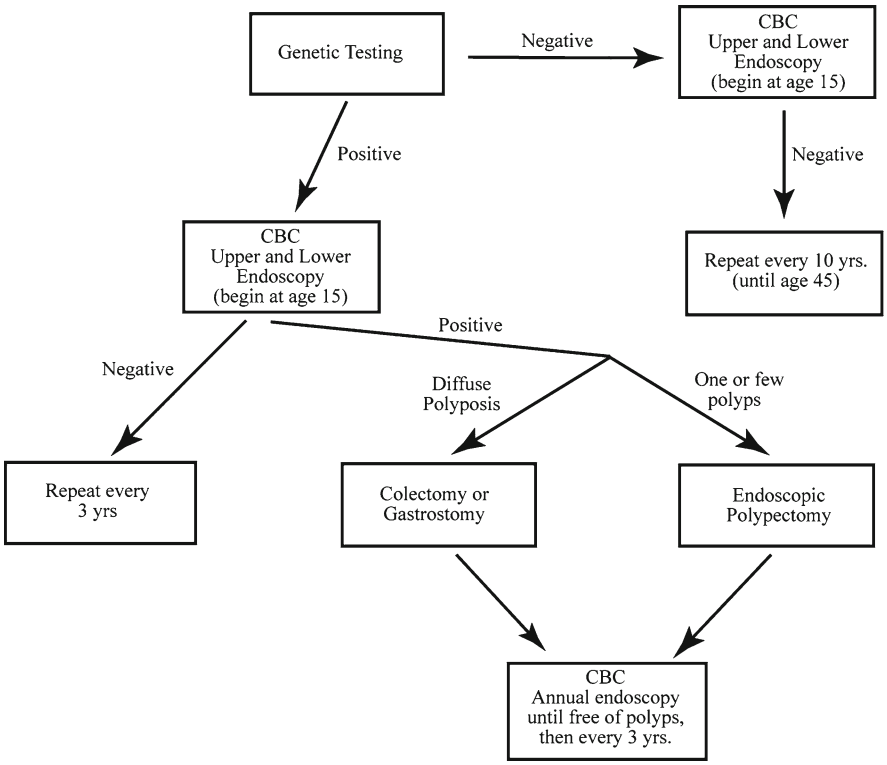

Fig. 21.6 Recommendations for screening and management in various groups at risk for JP (from Howe et al. [83], by permission of *Surgery*)

of the juvenile polyps; (2) resection of the most significantly affected areas of the bowel, usually indicated for severe blood loss requiring transfusions, diarrhea with significant weight loss or cachexia, signs of intussusception, or small bowel obstruction [19]. In 1984, Jarvinen et al. presented six patients with JP and polyps having foci of adenocarcinoma. They performed subtotal colectomy with ileorectal anastomosis and recommended regular follow-up starting at the age of 20 for JP patients due to the high risk of cancer, and screening of all family members [43]. Various authors have supported the view that JP patients should undergo regular evaluations with EGD and colonoscopy with polypectomy when necessary, even in patients with solitary juvenile polyps, although these patients may not require regular screening [20, 21, 24, 46].

21.8.2.2 Colectomy with Ileorectal Anastomosis

In 1986, Grosfeld et al. described a family with JP, which included five affected individuals who underwent surgery. Four patients had subtotal colectomy with ileorectal

anastomosis. Two patients had persistent anemia preoperatively and debilitating protein losing enteropathy causing failure to thrive. Another had recurrent intussusception, massive rectal bleeding, prolapse, and failure to thrive. One other had emergency laparotomy with segmental resection of a non-reducible intussusception of the splenic flexure and descending colon. They concluded that subtotal colectomy with ileorectal anastomosis was the procedure of choice in selected patients. The indications they put forth for surgery were children with anemia from chronic bleeding, hypoproteinemia, failure to thrive, and non-reducible intussusception [23].

Jarvinen et al. recommended prophylactic colectomy with ileorectal anastomosis for the surgical management of JP in 1993. Their indications were: (1) children with JP who have severe or repeated bleeding, which they believed might lead to failure to thrive or even death; (2) adults with JP due to the high cancer risk, which would exceed 50% in their lifetime. They felt the optimal timing of surgery in this group was at age 20–25 years since the cancer risk becomes more significant with age [79]. Howe et al. also recommended treatment with subtotal colectomy to remove the majority of the colon at risk, followed by evaluation of the rectal remnant every 1–3 years with flexible sigmoidoscopy and polypectomy as needed [47].

We feel that since many patients with JP may only have a few polyps at any one time, which can generally be removed endoscopically, surgery should be reserved for patients who have: (1) greater than 100 polyps or diffuse polyposis; (2) recurrent anemia requiring transfusions; (3) protein–losing enteropathy; (4) non-reducible intussusception. One negative consequence of aggressive surgical management includes the potential for frequent, uncontrolled bowel movements that can lead to social embarrassment, negatively impacting on psychosocial development in children. Frequent surveillance with colonoscopy and polypectomy appears to be a better choice for those with generally few polyps.

21.8.2.3 Total Colectomy with Ileoanal Pull Through

It has been estimated that the recurrence rate of solitary juvenile polyps is between 3 and 18%, which are primarily seen in patients <14-years-old [23]. In patients with JP, the recurrence rate is greater than 85%, and polyps continue to develop in the adult years [23]. Many authors have noted this trend of recurrence after JP patients undergo colectomy [19, 38, 41, 80, 81]. In 1995, Scott-Conner et al. performed a retrospective review of kindred consisting of 34 living members where 11 of them were diagnosed with JP. Eight patients underwent subtotal colectomies with ileorectal anastomosis. Two patients also had coexisting carcinoma of the stomach. Follow-up was done with colonoscopy and EGD, and polyps recurred in the rectal remnant of three patients at a mean of 36 months after surgery. Two patients underwent conversion to total proctocolectomy with ileoanal anastomosis and J-pouch, and in one patient, polyps recurred in the ileal reservoir 40 months later. Due to these findings, they recommended total colectomy with J pouch ileoanal anastomosis as the procedure of choice [81].

21.8.2.4 Operative Management for Gastric Disease

Many authors have concluded that individuals with generalized JP should undergo periodic surveillance of the upper GI tract due to the risk of gastric carcinoma [17, 43, 47, 48, 79, 81, 82]. If on screening, there are no abnormal findings, EGD should be repeated every 2–3 years [81]. Howe et al. recommended EGD to screen the upper GI tract in individuals at risk starting at age 15 as a baseline screening, or when signs and symptoms of upper GI involvement develop. If there are no gastric polyps, EGD can be repeated every 3 years. If there are polyps, EGD should be repeated yearly to evaluate the polyps for dysplasia. If there are complications such as bleeding, gastric outlet obstruction, dysplasia, adenomatous changes, or adenocarcinoma, then subtotal or total gastrectomy are indicated, depending on the location and extent of disease. Polypectomy is generally much more difficult in the stomach due to the diffuse nature of involvement of the gastric mucosa [47]. Patients with *SMAD4* mutations are at the greatest risk for gastric cancer [49, 50], and therefore will require particularly close attention.

21.9 Genetic Counseling

Two genes have been identified that cause JP, and this has made genetic testing of individuals at risk possible. In 1999, Howe et al. published a paper where the issue of genetic testing for *SMAD4* mutations in asymptomatic patients at risk for JP was addressed. Fifty five members of two JP kindred were analyzed via direct sequencing for *SMAD4* mutations. Their conclusions were that the genetic diagnosis of non-carrier status would allow for less frequent screening, such as that recommended for the normal population. In addition, patients who do have a mutation can be selected for closer endoscopic follow-up [83]. Howe et al. found that germline mutations of *SMAD4* and *BMPR1A* accounted for 40% of JP cases [37], and therefore, genetic testing can help to determine the recommended interval for screening and surveillance. The discovery of these two JP genes involved in JP has helped clinicians with screening of patients at risk of developing JP, and diagnosis at increasingly earlier ages. This should lead to closer attention to mutation carriers, and hopefully prevention of malignant transformation by early screening and polypectomy. Non-mutation carriers can be spared frequent endoscopic screening.

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Chapter 22
Hereditary Mixed Polyposis Syndrome and Multiple Adenoma Patients

Huw Thomas

Abstract Hereditary mixed polyposis syndrome (HMPS) is an extremely rare condition characterized by early age onset colorectal cancer and a variety of colorectal polyps of mixed histology. The genetic defect has been mapped in several Ashkenazi kindreds to chromosome 15q. In this chapter HMPS will be discussed.

Keywords Hereditary • Mixed • Polyposis Syndrome • Multiple • Adenoma • Patients

22.1 Introduction

Hereditary mixed polyposis syndrome (HMPS) is a rare condition in which individuals develop colorectal polyps and early onset colorectal cancer. Genetic studies in several Ashkenazi kindreds have mapped the gene to chromosome 15q [1, 2, 2A]. These kindreds share a common haplotype which is probably derived from a common founder, suggesting that the same gene is mutated in each kindred. There is currently no good evidence to show that HMPS occurs outside the Ashkenazi population. Other families that develop multiple colorectal adenomas, but often do not have germline mutations in the known predisposition genes, are also likely to have disease that is genetic in origin.

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22.2 Hereditary Mixed Polyposis Syndrome

22.2.1 Clinical Features

HMPS is an autosomal dominant syndrome characterised by the development of a variety of colorectal polyps of mixed histological type and colorectal cancer. The condition was first described in a large Ashkenazi kindred of Lithuanian origin. The proband presented to St Mark's Hospital in London in 1956, aged 28 with a six-month history of bright red rectal bleeding and lower abdominal colic [3]. Rigid sigmoidoscopy revealed multiple polyps in the rectum and a provisional diagnosis of familial polyposis coli was made on the grounds that several of the patient's close relatives had died of colorectal cancer at a relatively young age. However, the extracolonic features commonly associated with polyposis, such as sebaceous cysts and osteomas, were not present. Colectomy and ileo-rectal anastomosis were performed. The colectomy specimen revealed only six polyps, which was in contrast to FAP, where hundreds of polyps are usually present throughout the entire colon. Five of the six polyps were tubular adenomas and one was a juvenile-type polyp with overlapping adenomatous and hyperplastic histological features. The patient remains asymptomatic in 2007 with the occasional development of rectal polyps that are removed at colonoscopy.

Many members of this kindred, SM96, have since been ascertained through the proband and several additional HMPS kindreds have now also been described, all of which are of Ashkenazi origin [2]. Older individuals in these families present with colorectal carcinomas while a colonoscopic surveillance of younger members can reveal up to 50 polyps distributed throughout the large bowel. The number and histology of polyps vary between patients but include atypical juvenile and hyperplastic polyps and adenomas, including those of serrated appearance; many of the tumours have mixed histological features (see Table 22.1) [3, 4]. Serrated adenomas occur at a frequency as low as 1% in unselected tumour populations [5, 6], but in SM96 their frequency is significantly higher at approximately 14% (unpublished). The tumour spectrum suggests that progression can occur from juvenile/hyperplastic polyp to mixed/serrated adenoma to carcinoma.

Table 22.1 Variety of polyps identified when 84 polyps from 10 members of SM96 were recently reviewed (unpublished)

Histology of polyp	Number
Hyperplastic polyp	16
Inflammatory polyp	1
Juvenile polyp with mixed features	8
Serrated adenoma	12
Tubular adenoma	41
Tubulovillous adenoma	5
Villous adenoma	1

22.2.2 Genetic Studies in HMPS

22.2.2.1 Linkage Analysis

The Adenomatous Polyposis Coli gene together with *MLH1, MSH2, MSH6, TP53, DCC* and a variety of other loci were excluded as candidates for HMPS, by mutation screening and the absence of genetic linkage [1, 7]. This information provides support that HMPS is a distinct syndrome.

An initial genomewide linkage study carried out on SM96 mapped the *HMPS* gene to 6q16-q21 [7]. However, subsequent to this, a family member not carrying the disease-associated haplotype developed multiple colorectal adenomas before the age of 40, strongly suggesting that the linkage to chromosome 6 was incorrect. The affection status of family members was therefore reassessed and updated using more stringent criteria, while linkage to chromosome 6 was retested and a new genomewide linkage screen undertaken [2]. These studies demonstrated no evidence of linkage to chromosome 6, but revealed one site in the genome with good evidence of linkage to HMPS. Haplotype construction showed this region to be a 10 cM interval located on chromosome 15q13-q21 (Fig. 22.1). A genomewide study previously performed on another Ashkenazi family (SM1311) had mapped a new colorectal tumour susceptibility gene, *CRAC1,* to 15q14-q22 [1]. Further investigation showed that affected members of SM1311 shared the same haplotype as SM96 for the 10 cM region on 15q13-q21. In addition, the disease-associated haplotype was also found to be present in a further three Ashkenazi kindreds with multiple colorectal polyps. The haplotype was rare in the general Ashkenazi population, being absent in any spouse marrying into the families studied, and present in at most, 1 of 95 random Ashkenazi controls. The location of the *HMPS* locus was therefore predicted to lie on chromosome 15 in a region between 29,875,416 and 34,024,377 (UCSC genome browser, 2006 build; http://genome.ucsc.edu).

The phenotypes of the five families were very similar, with members developing multiple, classical colorectal adenomas and carcinomas. Members of families SM96, SM1311, and RF also developed polyps with mixed hyperplastic/adenomatous features and serrated adenomas, while SM96 members were the only individuals to develop atypical juvenile polyps.

With the evolution of modern SNP array technology, it was possible to fine-map and reduce the minimal region further [2A]. Eight selected, affected individuals (probands and other family members previously shown to have critical recombinations) and one unaffected, non-carrier mother of a patient, were genotyped and a minimal shared region on chromosome 15 identified. The location of the *HMPS* gene was therefore further restricted to chr15:30,735,098-31,369,755. This region contains three known genes: the 3′ part of *SGNE1/SCG5*; *GREM1/DRM/CKTSF1B1;* and *FMN1.* In addition, hypothetical genes C15orf45, AX747968 and DKFZp686C2281 map to the region. Despite sequencing all coding sequences, introns, promoter regions and other highly

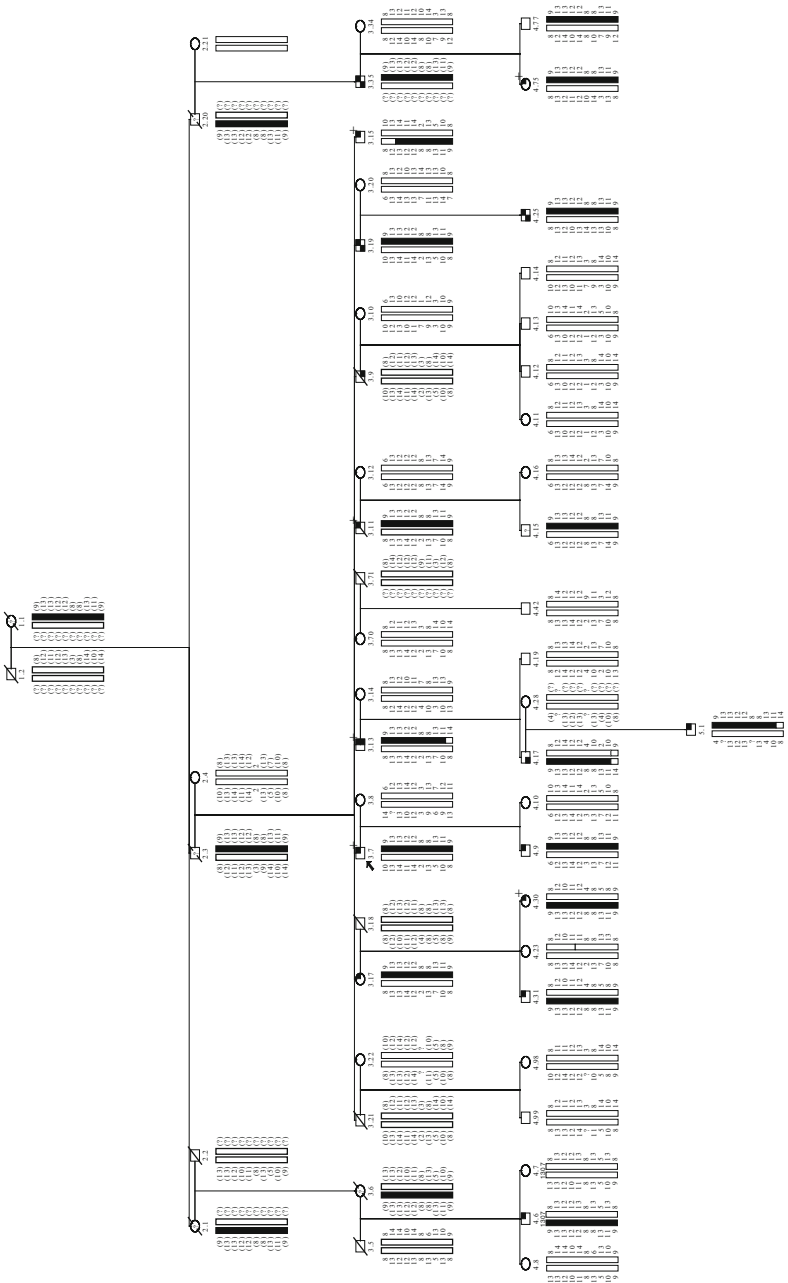

Fig. 22.1 Pedigree of selected members of family SM96, showing haplotypes for the following chromosome 15 markers: D15S1031, D15S1010, D15S144, D15s995, D15S1007, D15S1040, ACTC, D15S971, and D15S118. Known affected individuals are indicated by a *blackened symbol*. Inferred haplotypes are *bracketed*

conserved regions within the minimal region, no mutations unique to HMPS patients have been identified [2].

22.2.2.2 Loss of Heterozygosity

To investigate the possibility that *HMPS* might act as a tumour suppressor, Loss of heterozygosity (LOH) analysis was performed on tumours from SM96 and SM1311 [1, 2], resulting in limited evidence for LOH. The reasons for the low frequency of LOH – for example, dominant germline mutation, haploinsufficiency or "second hit" by promoter methylation – are as yet, unclear.

22.2.2.3 Association Study

Genetic association studies have provided the opportunity to test whether the high-penetrance *HMPS/CRAC1* locus might also harbour common low-penetrance variants that increase the risk of colorectal cancer in the general population. A hundred and forty-five tagging SNPs were genotyped in 718 colorectal cancer cases selected for family history and/or early onset disease and in 960 controls from the UK [2A]. Two SNPs, rs4779584 and rs10318 located at chr15:30,782,048 and 30,813,271 respectively, showed associations with disease. These SNPs were therefore genotyped in another three large cohorts of similar colorectal cancer cases and controls, providing a total of 7,961 successfully typed cases and 6,803 controls. Overall, both SNPs were very strongly associated with disease: for rs4779584, $P = 4.44 \times 10^{-14}$ under the allele test and for rs10318, $P = 7.93 \times 10^{-14}$, with corresponding odds ratios of 1.26 (95%CI 1.19–1.34) and 1.19 (95%CI 1.12–1.26) respectively. No evidence was found to suggest that each SNP contributed independently to disease risk. Sequencing of coding regions, UTRs, splice junctions, reported transcripts, reported control regions and other highly conserved regions in 92 UK familial colorectal cancer cases did not reveal any obvious disease-causing variants.

The SNP rs10318 is located within the 3′UTR of *GREM1,* a secreted bone morphogenetic protein (BMP) antagonist. Although no obviously pathogenic *GREM1* variant was identified, the TGF-beta/BMP pathway is known to play an important role in colorectal tumorigenesis; *BMPR1A* mutations, for example, cause juvenile polyposis, a disease typified by lesions that resemble HMPS polyps. It is therefore possible that GREM1 may increase tumour proliferation, for example, through its expression in the stroma [8]. Rs4779584 lies between *GREM1* and *SGNE1*. Neuroendocrine signalling involving *SGNE1* could influence cellular proliferation in the large bowel through, for example, signalling of nutrient availability or through systemic effects [9]. Work is currently ongoing to identify the causal variant of HMPS.

22.2.3 Clinical Management of HMPS

There is no evidence for extra-colonic tumours in the HMPS pedigrees, nor evidence of accelerated tumorigenesis as seen in Lynch Syndrome. We recommend 5-yearly colonoscopic surveillance initially in at-risk family members from the age of 25, increasing to 3 yearly if polyps are found. Affected individuals can be managed by colonoscopic polypectomy and rarely require colectomy unless they develop a colorectal cancer. Genetic testing is not yet available and although a prediction or diagnosis can be made with some confidence based on the disease haplotype and clinico-pathological features, we consider this not currently of sufficient reliability for routine use.

22.3 Multiple Adenoma Patients

22.3.1 Clinical Features

Multiple colorectal adenomas are found in some individuals with a personal or family history of colorectal cancer. Individuals with more than five colorectal adenomas form a heterogeneous group, some of whom may have a family history of colorectal neoplasia. Approximately 6% of at-risk individuals with a dominantly inherited predisposition to colorectal cancer in whom FAP and HNPCC/Lynch syndrome have been excluded develop five or more colorectal adenomas [10]. The definition of a multiple adenoma case is not yet established, but one possible diagnostic scheme is as follows.

1. Set a practical working lower limit on adenoma number (generally ten, although sometimes five is used, especially where the case is of early onset, say <45 years).
2. Exclude *APC*, *MYH* and (generally for lower (<~10) polyp numbers and/or suggestive family history) MMR (HNPCC) mutations.
3. Consider any individual with more than 1,000 adenomas as having presumed FAP (or possibly *MYH*-associated polyposis), taking into account all molecular and clinico-pathological evidence.
4. Consider any individual with more than 100 adenomas as having probable FAP or *MYH*-associated polyposis, taking into account all molecular and clinico-pathological evidence.
5. Regard the remainder of cases as “multiple adenoma patients.”

Overall, 5–10% of cases with 5–100 adenomas at presentation are due to dominantly inherited *APC* mutations (causing attenuated FAP) and a further 30% of individuals have bi-allelic *MYH (MUTYH)* mutations(causing the recessive condition MYH-associated polyposis (MAP)). Attenuated FAP and MAP are discussed in separate Chapters.

22.3.2 Genetic Studies

Laken et al. [11] reported an individual with eight colorectal adenomas and a family history of colorectal cancer in whom the diagnosis of HNPCC/Lynch syndrome had been excluded. They demonstrated a germline T-to-A transversion at nucleotide 3920 of the *APC* gene. This mutation produces a small hypermutable region of the gene with somatic mutations leading to the development of adenomas and subsequently colorectal cancers in these individuals. This variant is associated with a twofold increased risk of colorectal cancer. As yet, this alteration has only been described in individuals of Ashkenazi Jewish origin and occurs at a frequency of about 6% in this group. Further germline variants of the *APC* gene have been sought in individuals with multiple colorectal adenomas and carcinomas. A G to C point mutation at codon 3949 (E1317Q) has been described in four individuals with multiple adenomas, and in three was also associated with multiple colorectal hyperplastic polyps and in one with colorectal cancer [12]. Currently, however, a convincing pathogenic effect of E1317Q cannot be established and it should not be used for diagnostic or predictive testing.

Thirlwell et al. [13] investigated 25 individuals with multiple colorectal adenomas in whom germline mutations of the *APC*, *MYH* and mismatch repair genes had been excluded. No vertical transmission of the multiple colorectal adenoma phenotype was found and there were no associated extracolonic cancers. Somatic mutations within the adenomas were similar to those found in sporadic colorectal neoplasia and did not show evidence of a specific mutational signature as seen with defects of mismatch or base-excision repair. It is possible that some multiple adenoma cases result from a combination of low-penetrance alleles, but the sheer number of polyps, often tens of lesions in some cases, leaves open the possibility that a high-penetrance predisposition gene awaits discovery. No obvious planned route to finding this gene exists, although whole-genome sequencing is an attractive prospect.

22.3.3 Clinical Management of Multiple Adenoma Patients

Individuals with multiple colorectal adenomas may usually be managed by colonoscopic polypectomy. Clearance of all the polyps should be attempted. When this has been achieved, regular surveillance colonoscopy is required, the frequency of which may depend on the number of new polyps developing. Currently, in the absence of a clear phenotypic characterisation, pragmatic titration of screening intervals to the individual is necessary. Surgery may be required if the patient presents with carcinoma or overwhelming numbers of new polyps develop. Multiple adenoma patients do not appear to exhibit the accelerated tumour progression seen with germline alterations of mismatch repair genes. This is a heterogeneous condition and, although most cases appear to be isolated, a family history of colorectal neoplasia is not infrequently present. It would therefore be prudent to suggest that first-degree relatives also consider surveillance colonoscopy.

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Part VIII
Clinical Science: Hyperplastics Polyposis

Chapter 23
Hyperplastic Polyposis

Angel Ferrández and Randall W. Burt

Abstract Hyperplastic polyposis is characterized by multiple or large hyperplastic polyps. Recent studies suggest a substantial rate of colorectal cancer. In this chapter, the clinicopathologic characteristics, management, and surveillance recommendations will be reviewed.

Keywords Hyperplastic polyposis • Hyperplastic • Polyposis • Colon cancer

23.1 Introduction

Hyperplastic polyposis is a rare condition in which patients present with multiple or large hyperplastic colonic polyps. It is generally asymptomatic and its diagnosis is based on clinical criteria as defined by the World Health Organization (WHO) classification of tumors. It has been considered a benign condition with no malignant potential, but recent studies have shown a substantial rate of colorectal cancer development. Additionally, several different genetic alterations have been suggested in such neoplastic progression. Surveillance colonoscopy, every 1–3 years with polypectomy of as many polyps as possible has been recommended, although some patients may need prophylactic colectomy.

23.2 Definition and Diagnostic Criteria

Hyperplastic poliposis is a rare condition in which the colonic mucosa harbors multiple or large hyperplastic polyps. It was first described by Williams et al. in 1980 [1] although two similar cases had been reported previously [2–4]. Williams

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reported seven cases with more than 50 hyperplastic polyps in each patient that caused diagnostic confusion with familial adenomatous polyposis. Those patients were followed for periods of 3–13 years and none developed colorectal malignancy, so the condition was considered to be without malignant potential. Since then, a number of authors have reported cases of this hyperplastic polyposis (Table 23.1). Several cases were also found to have colorectal carcinoma, raising the possibility of a relationship of this condition to colon cancer.

In 1984, Urbanski et al. introduced the term mixed hyperplastic–adenomatous polyp to describe polyps with characteristics of both histological types [5]. This concept was more rigorously described several years later by Longacre et al. [6] and lesions were renamed serrated adenomas, emphasizing their neoplastic nature. Serrated adenomas have been reported in hyperplastic polyposis. While one study proposed renaming this syndrome serrated adenomatous polyposis [7], other studies have not found serrated adenomas to be uniformly present with hyperplastic polyposis [8].

The hyperplastic polyposis syndrome is generally asymptomatic and discovered at screening colonoscopy. Large hyperplastic polyps occurring in the syndrome may occasionally bleed, however, leading to the diagnosis in this way. Although hyperplastic polyposis is considered rare, the lack of specific symptoms makes its under-diagnosis quite possible.

Small hyperplastic polyps are often endoscopically indistinguishable from diminutive adenomas. Thus distinguishing hyperplastic polyposis from familial adenomatous polyposis may be difficult without histopathology. New endoscopic technologies, however, including chromoendoscopy, endoscopic magnification, and narrow band imaging (NBI) could help the endoscopist in this task. Both low-magnification and high-magnification NBI are capable of distinguishing neoplastic from non-neoplastic colorectal lesions. The diagnostic accuracy of NBI has been demonstrated to be better than that of conventional colonoscopy and equivalent to that of chromoendoscopy [9].

Recently the publication, "World Health Organization (WHO) International Classification of Tumors [10]," suggested a working definition of hyperplastic polyposis as follows:

1. At least five histologically diagnosed hyperplastic polyps proximal to the sigmoid colon, of which two are greater than 10 mm in diameter.
2. Any number of hyperplastic polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with hyperplastic polyposis.
3. Greater than 30 hyperplastic polyps of any size, but distributed throughout the colon.

However, not all the authors agree with this definition, particularly with the third statement, and some have used the presence of 20 hyperplastic polyps of any size throughout the colon as a definition of this syndrome [8].

The WHO working definition was an attempt to unify criteria for the diagnosis of hyperplastic polyposis. This definition was based, in part, on the somewhat arbitrary definition by Jorgensen [11] who proposed that patients should be included in this category if they had at least 20 hyperplastic polyps distributed evenly or segmentally in the colorectum and/or at least two hyperplastic polyps

Table 23.1 Hyperplastic polyposis studies according to the WHO classification and patient characteristics

Author	References	Cases	Age (mean)	Adenoma	Carcinoma	Location of cancer	Family history	Number of hyperplastic polyps
Hayakawa K.	[37]	–	–	–	–	–	–	–
Goswami	[38]	1	64	–	Yes	RC	No	–
Cooke SA.	[3]	1	–	Yes	No	–	No	30
Cooper HS.	[2]	1	–	Yes	Yes	RC	Not stated	Multiple
Franzin G.	[39]	1	–	No	In situ	–	–	1
Urbanski SJ.	[5]	1	–	Yes	In situ	–	–	1
Bengoechea O.	[40]	1	24	Yes	Yes	RC	No	Multiple
McCann BG.	[41]	1	Elderly	Yes	Yes	RC	Not stated	>60
Teoh H.	[42]	1	76	Yes	Yes (4 metachr.)	LC	No	18
Warner AS.	[43]	1	56	Yes	Yes (in situ)	Not specified	No	16
Orii S.	[24]	1	55	Yes	Yes	Not specified	No	200
Keljo DJ.	[44]	1	11	Not reported	Yes (11 year old)	R	No	>100
Hawkins NJ.	[45]	1	–	Yes (serrated)	Yes (in 2: synchr.)	LC	–	75
Lieverse RJ.	[46]	1	–	Yes	Yes	LC	–	Multiple
Sumner HW.	[47]	1	–	Yes	No	–	–	>100
Beusnel C.	[48]	1	–	Yes	No	–	–	Multiple
Koide N	[25]	1	–	Yes	Yes	RC	–	Multiple
Cohen SM.	[49]	1	26	No	No	–	No	–
Tulman AB	[50]	1	–	No	No	–	–	15
Kusunoki M.	[51]	2	69	Yes	Yes (in 2)	RC	No	19–61
Shepherd NA	[52]	2	–	Yes (in 1)	No	–	–	12–18
Jorgensen H.	[53]	3	47	No	Yes (in 2)	LC	No	Multiple – 68
Jass JR.	[27]	4	–	Yes (all)	Yes (all)	RC, 3; LC, 1	–	6–50
Franzin G.	[54]	5	–	Yes (in 4)	Yes (in 1)	–	–	14

(continued)

Table 23.1 (continued)

Author	References	Cases	Age (mean)	Adenoma	Carcinoma	Location of cancer	Family history	Number of hyperplastic polyps
Jeervaratnam P	[16]	5	–	Yes (serrated)	Yes (all)	–	–	Multiple
Place RJ.	[55]	6	61	Yes	Yes (in 3)	RC, 2; LC, 1	No	>100
Torlakovic	[7]	6	29	No	No	–	Not stated	Multiple
Oberchsmid	[15]	6	61	No	Yes (all)	RC, 3; LC, 3	Not stated	WHO criteria
Williams GT.	[1]	7	–	Yes	No	–	1/7	50–156
Spjut	[4]	9	–	Yes (in 1)	Yes (in 1)	Not specified	–	Multiple
Rubio	[56]	10	61	Yes (in 8)	Yes (in 7)	RC, 4; LC, 1; R, 2	1/10	7–159
Leggett BA.	[26]	12	–	Yes (in 11)	Yes (in 7)	Not specified	–	>15 to >100
Rashid A.	[8]	13	–	Yes	Yes (in 10)	RC, 2; LC, 8	2/13	Multiple – >100
Hyman	[36]	13	60	Yes (in 10)	Yes (in 7)	RC, 5; LC, 1; R, 2	5/13	30 – >100
Lage	[17]	14	69	–	Yes (in 6)	RC, 4; LC, 2	7/14	19 – >100
Ferrández	[20]	15	53	Yes (in 11)	Yes (in 1)	Not specified	None	16–210
Chow	[18]	38	52	Yes (in 30)	Yes (in 10)	RC, 4; LC, 5; R, 1	2/38	3 – Multiple
Total (>5 cases)		159		11/13 studies	64 (40.2%) patients		18/116	

larger than 10 mm in diameter. Both definitions are arbitrary and it is likely that when molecular and genetic mechanisms underlying this condition are better understood, a better definition and description can be provided. A review of the literature about hyperplastic polyposis is shown in Table 23.1.

Hyperplastic polyps are a common finding in the colon and rectum. It has been estimated that approximately 85% of North Americans will develop at least one hyperplastic polyp by age 65 years. The vast majority are small and tend to be located in the distal colon [12]. However, their importance as a precursor for colorectal cancer is still controversial. Most investigators consider hyperplastic polyps to be an incidental finding with no potential for progression to colorectal cancer [13], while others contend that hyperplastic polyps are precursors of colorectal adenomas and cancer [11]. In fact, hyperplastic polyps share epidemiologic and colonic distribution characteristics with colorectal adenomas and cancers [14].

Another important consideration concerning the phenotype of hyperplastic polyposis is that many of those affected exhibit one or several concomitant colonic adenomas at the time of diagnosis. In fact, in 11 out of the 13 studies in which more than five patients were reported, adenomas were found together with the hyperplastic polyposis. This observation adds a risk of colorectal cancer to this condition separate from the hyperplastic polyps themselves.

In contradistinction to the near ubiquitous observation of several small distal hyperplastic polyps, patients with hyperplastic polyposis are demonstrably unique in view of the frequency, distribution, and often large size of the hyperplastic polyps observed [8]. The hyperplastic polyps found in hyperplastic polyposis also have different genetic characteristics compared to sporadic hyperplastic polyps. They exhibit, for example, secreted Frizzled receptor protein 1 immunophenotype, which could indicate alterations of cellular growth control [15].

Familial aggregation of hyperplastic polyposis has been occasionally observed. This observation has led to the hypothesis that this condition is inherited. Nonetheless, evidence for inheritance remains scant. As seen in Table 23.1, when only those papers with five or more cases of hyperplastic polyposis are considered, only 15.5% (18/116) of the patients have even had a first degree-relative with colorectal cancer. Furthermore, only few cases have been reported with an actual family history of hyperplastic polyposis and colorectal cancer [8, 16–18]. These case reports indeed suggest that inherited predisposition may sometimes occur, but the specific genes involved in such inheritance have not been identified.

There is increasing evidence that hyperplastic polyposis could be the phenotypic expression of several different genetic alterations. Three different pathways have been proposed:

1. The hyperplastic polyp–dysplasia/adenoma–adenocarcinoma sequence involving K-ras, p53, MSI, and loss of 1p was proposed by Rashid et al. [8] based on genetic findings in a few admixed hyperplastic–adenomatous polyps (lesions related to hyperplastic polyps, with epithelial dysplasia).
2. Another pathway is suggested in patients with a large hyperplastic polyp in the right colon. Such patients also appear to have an increased risk of right-sided

colon cancer. In the hyperplastic polyps of such patients, frequent topographic dysregulation of p21Waf-1/Cip1 and Ki-67 are observed, although there is a lack of K-ras mutations.

3. A distinctive third pathway is manifested in patients with more than 20 hyperplastic polyps. It is characterized by frequent allelic loss of chromosome 1p, but a lower frequency of K-ras mutations, p53 over expression and MSI in hyperplastic polyps. In this group, family members with colorectal cancer are also sometimes observed.

A recent study has suggested that a genetic predisposition may underlie at least some forms of hyperplastic polyposis in which the earliest manifestation may be the hypermethylation of multiple gene promoters in normal colorectal mucosa [19]. In fact, it seems that there is more extensive methylation in sessile serrated adenomas from subjects with hyperplastic polyposis compared to matched normal mucosa ($P<0.0001$). A more clear-cut difference in patients with hyperplastic polyposis was the finding of extensive DNA methylation in normal mucosa from the proximal colon. It has also been proposed that some of the heterogeneity within hyperplastic polyposis may be explained by different propensities for *MLH1* inactivation within polyps.

23.3 Hyperplastic Polyposis and Colorectal Cancer

Little is known about the risk of cancer development in hyperplastic polyposis. If the studies with more than five patients with hyperplastic polyposis are analyzed separately, 40% of such patients are diagnosed with colorectal cancer. But as mentioned before, this condition is probably under-diagnosed. Many cases with hyperplastic polyposis and no cancer probably go undiagnosed. Cases that are identified and published may well represent ascertainment bias toward those more severe phenotypes and toward those presenting with colorectal cancer.

Moreover, there is no available information on the risk of colorectal cancer during the surveillance of hyperplastic polyposis. In the group of 15 patients from the Huntsman Cancer Institute reported by Ferrández et al. [20], prophylactic colectomy was recommended in three because of the presence of high-grade dysplasia during surveillance (unpublished observations by Dr Burt). When more data from established polyposis registries become available, the true risk of neoplastic progression and/or association in hyperplastic polyposis patients could be clarified.

As mentioned previously, small distal colonic hyperplastic polyps have been typically considered an innocent finding with no potential for progression to colorectal cancer. Considering the WHO definition of hyperplastic polyposis, two major phenotypes can be distinguished: (1) patients with multiple small polyps usually with more polyps in the distal colon and (2) those with small numbers of large and mainly proximal polyps. Recent literature strongly supports that large and proximal hyperplastic are morphologically and genetically distinct from the first category, as well as from sporadic hyperplastic polyps. The morphologic heterogeneity of hyperplastic

polyposis may explain why some studies have failed to show a convincing link with colorectal cancer [1, 20] while others have shown such an association.

However, it has become increasingly clear that there indeed exists a degree of genetic and perhaps morphologic heterogeneity among hyperplastic polyps. And despite similar histologic appearances, there seems to be a subset of hyperplastic polyps that may carry a significant risk of progressing to colorectal cancer, or at least serve as a marker for the development of colorectal cancer [15, 21–26].

Investigations that have focused on right-sided hyperplastic polyps have suggested that these may be the precursors of the subset of colorectal cancers that exhibit high-level DNA microsatellite instability (MSI-H). This seems to be the case in proximal colonic hyperplastic polyps that occur both as a part of hyperplastic polyposis [27] and those that occur sporadically [21, 28–30]. However, this observation has not always been consistent. Patients, for example, with colorectal cancer complicating hyperplastic polyposis have been found to have clinical features of MSI-H cancers such as early age of onset, multiplicity, and proximal colon location. But the cancers themselves have been observed to be either MSI-H or MSI-low or MSI-stable (MSS). In fact, in the largest series of hyperplastic polyposis where polyps and cancers were examined for MSI, testing was done in five polyps and four cancers. Interestingly, all tumors tested were MSS [18]. But the heterogeneity of MSI testing results appears to be high in all the series.

Hyperplastic polyposis as a syndrome may also carry a very significant risk for the development of colorectal cancer. Jass and colleagues have described dysplastic transformation in a small subset of hyperplastic polyps, which they have linked with a pathway of colorectal neoplasia characterized by DNA metaplasia and microsatellite instability [7, 30, 31]. A wide variety of molecular abnormalities characteristic of colorectal neoplasia have been shown by others to occur in hyperplastic polyps [27–29]. It has been proposed that hyperplastic polyps may serve as the initial lesion in a serrated neoplasia pathway that results in the 15% of sporadic colorectal adenocarcinomas that are microsatellite unstable [32]. A recent study has suggested morphologic criteria to identify the subgroup of hyperplastic-like polyps that serve as the initial lesion in this serrated neoplasia pathway [32].

23.4 Management of Patients with Hyperplastic Polyposis

There are no consistent guidelines on the management of patients with hyperplastic polyposis and their relatives, and those given are based on expert opinion rather than prospective investigation. The current suggested endoscopic surveillance recommendation for an individual with hyperplastic polyposis is every 1- to 3-yearly colonoscopy examinations, depending on the number, size, and histology of polyps [20].

Although it may be impossible for the gastroenterologist to remove every minute polyp, especially those tiny polyps in the distal colon and rectum, special attention should be paid to polyps with clinical and endoscopic features that indicate “high-risk” hyperplastic polyps. These features include multiple polyps (>20),

large polyps (>10 mm), and proximal polyp location. A family history of colorectal cancer also appears to be important. All proximal colonic polyps should be resected completely because adenomatous tissue may be present and some polyps may be serrated adenomas [33].

Surveillance following initial polypectomy is warranted, and although the optimal frequency is unclear, it has been suggested that patients with "high-risk" hyperplastic polyps or serrated adenomas be approached in the same way as patients with adenomas [34].

Clinicians should count the number of polyps each time a patient has a colonoscopy and submit all polyps for histologic examination if possible. It is also important that the endoscopist keep a running total of the number, size, location, and histopathology of all previous polyps in order to better provide appropriate management. Pathologists should be familiar with the characteristics of serrated adenomas, including sessile-serrated polyps and admixed hyperplastic–adenomatous polyps, which seem to be highly discriminative for hyperplastic polyposis.

Screening colonoscopies should be recommended for first-degree relatives of affected individuals, independent of the presence of cancer in the proband. The appropriate age to begin screening and the screening interval in relatives remain unclear. The Australian National Health and Medical Research Council guidelines suggest screening first-degree relatives of those with hyperplastic polyposis with every 5-year colonoscopy [35]. The interval should be decreased if adenomas or multiple hyperplastic polyps are detected. The starting age for screening in relatives should be 40 years or 10 years earlier than the earliest age of polyposis diagnosis in the family.

Other guidelines recognize the risk of developing adenomas and colorectal cancer in hyperplastic polyposis patients and recommend biannual colonoscopy [36].

Colectomy may be justified when surveillance and control of the polyps becomes colonoscopically difficult. This would be in patients with a high number of hyperplastic polyps, especially if concomitant serrated adenomas, or multiple high-risk adenomatous polyps (more than 1 cm, villous component, high-grade dysplasia) are present [8]. Finally, if some hyperplastic polyps begin to develop areas of high-grade dysplasia, colectomy should probably be considered.

23.5 Conclusions

The syndrome of hyperplastic polyposis represents a challenge for the clinician. Its rarity has made the synthesis of precise surveillance and management guidelines for the patients and their relatives difficult. However, recent studies have provided useful information on the clinical presentation, the phenotypic characteristics and the risk of colorectal cancer in patients with this condition. Several genetic alterations observed in polyps have now been associated with malignant potential in hyperplastic polyposis patients. It is likely that ongoing genetic research will provide a better understanding of the possible inheritance, cancer risk and pathogenesis

of this condition. Such information will also allow for a precise diagnosis and phenotypic description of hyperplastic polyposis as well as the possibility of genetic testing in patients and their relatives.

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Part IX
Genetic Counseling

Chapter 24
Genetic Counseling Overview

Terri Berk

Abstract Family history may be the single most important aspect of treating patients with an inherited colorectal cancer syndrome. Clinicians face an uphill battle in learning to recognize the hereditary features of these syndromes and their implications. The International Society of Gastrointestinal Hereditary Tumours and the Collaborative Group of the Americas for Inherited Colorectal Cancer serve as resources for this service gap, recognizing the affected family as the "patient." Referral to a genetic register will benefit the clinician and the patient. The process of genetic counseling provides a framework for understanding the intricacies of predictive testing. The challenges of informed consent and assent by adolescents; interpretation of uninformative results; patient confidentiality; and psychosocial sequelae are addressed by genetic registers.

Keywords Genetic counseling • Inherited colorectal cancer syndromes • Predictive testing • Informed consent

Henry Lynch serves as a role model for counseling in inherited colorectal cancer. He popularized if not pioneered the concept of the Family Information Service by counseling families where they live, as part of the Creighton Cancer Genetic Research effort [1]. Recently, one of their HNPCC patients publicly acknowledged gratitude for the compassionate manner in which this research was conducted, and expressed the hope that one day scientists might find that the MSH2 gene was also the gene for courage. Let's review the rationale for genetic counseling in inherited colorectal cancer syndromes and a brief algorithm for the process.

Professional endorsements for predictive testing offer guidelines to health care professionals [2–5]. Online resources to find cancer genetics centers, take a family history, or locate a genetic counselor/laboratory/genetic register include:

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M.A. Rodriguez-Bigas et al. (eds.), *Hereditary Colorectal Cancer*, M.D. Anderson Solid Tumor Oncology Series 5, DOI 10.1007/978-1-4419-6603-2_24,

http://www.cancer.gov/cancertopics/pdq/genetics-directory-background;
http://www.hhs.gov/familyhistory;
http://www.nsgc.org/resourcelink.asp;
http://www.genetests.org;
http://www.insight-group.org;
http://www.cgaicc.com.

Since the identification of the APC gene in 1991, FAP is considered a role model for carcinogenesis. The National Comprehensive Cancer Network recommends genetic counseling for FAP, acknowledging the involvement of trained specialists [6]. However, since APC testing is available in service laboratories, patients or local health care providers may realistically obtain predictive testing without counseling or fully informed consent. A recent example of devastating psychosocial sequelae occurred after one family received positive APC results by mail for five at-risk children whose family physician explained the test as a "routine blood sample." The affected parent's suicide was compounded by problems with adolescent noncompliance with surveillance and substance abuse.

Children are most vulnerable as they contend with developmental issues during adolescence, which may challenge the reality of covert disease such as FAP [6, 7]. A pediatric framework for genetic testing tries to balance the rule of earliest onset for FAP and HNPCC so that children who assent are part of the decision making [8]. Longitudinal research suggests that many children who are counseled and undergo testing for APC are not distressed but that issues such as anxiety or depression are more prevalent in both mutation-positive and mutation-negative patients if they have an affected sibling [9]. Some FAP studies have found that the need for ongoing support exists and should not be restricted to adolescence [10]. Burn details the inner workings of a family cancer clinic as an alternative for risk assessment and long-term supportive counseling [11].

Clinicians are often the gatekeepers for predictive testing. Problems arise when surgical history taking is inadequate, particularly for HNPCC, and putative cases of inherited colorectal cancer are underestimated [12, 13]. Since most nonspecialists will be unfamiliar with the intricacies of current research criteria, errors can occur causing psychogenic costs that outweigh benefits. For example, in the 1997 APC testing survey of 177 patients, 20% of tests were performed on non-FAP patients; uninformative results were misinterpreted as a negative result by clinicians in close to one-third of the cases [14]. Indeed, there have been legal precedents for clinicians who do not pursue a "duty to warn" in FAP and HNPCC [15, 16]. Given these legal constraints and emerging technologies, posttest counseling sessions include an offer to patients to maintain contact with the counseling center [17].

It cannot be overstated that uninformative genetic results do not rule out clinical or pertinent family history but reflect a limitation of current technology and test sensitivity [18, 19]. Working hypotheses for clinicians about whom to test, test sensitivity, and cost estimates may be applied to all forms of inherited colorectal cancer, including hamartomatous and hyperplastic polyposes and MYH-associated polyposis (MAP) [19, 20]. Given the practical difficulties of ascertaining affected families, it falls to genetic registers such as the International Society for Gastrointestinal Hereditary

Tumours (InSiGHT) or the Collaborative Group of the Americas for Inherited Colorectal Cancer (CGA) to address this service gap. Awareness of current research applications such as the MSH2 founder mutation or new molecular studies for hyperplastic and mixed polyposes allows counselors to better educate patients [21, 22].

Although counseling sessions are tailored toward the individual, commonly discussed areas are background and ethnicity; social/medical/family history; self-concept and psychosocial resources; confirmation of clinical records and histopathology; heredity and risk factors; patient's perception of risk; surveillance and treatment recommendations; testing process and implications; available supports and follow-up contact information [20]. Direct sessions are encouraged before and after genetic testing, along with a letter summarizing the discussion each time so that the patient has something concrete to refer to over time [4].

Informed consent generally involves the use of a comprehensive, Institutional Research Board-approved form, describing the disease; purpose of the test; type of testing; meaning of positive and negative results; predictive value; and potential clinical options. Given the current climate of privacy constraints, many countries are governed by legislation such as the Personal Data Act, a European Union directive, or the Health Insurance Portability and Accountability (HIPPA), a US initiative [23, 24].

The thorny issue of disclosure of genetic information and patient confidentiality currently favors patient privacy superseding the right to know for at-risk relatives [4, 15]. This conflict may arise during the counseling session if patients refuse to share mutation status with family members [25]. Other studies have demonstrated that a majority of gene-positive and gene-negative patients are willing to share results with family after counseling and that doing so improved their relationships [26, 27]. Creative approaches encourage today's counselor to hone communication skills and new educational tools in diverse societies [28]. Genetic counselors with expertise in molecular research offer informed discussion about new technologies such as preimplantation diagnosis for FAP, as patients seek out reproductive options and learn about cost or insurance barriers [29]. The red flag of insurance discrimination is oft quoted as a deterrent to predictive testing, reflected in the 2000 survey of the National Society of Genetic Counselors Special Interest Group in Cancer. In a hypothetical genetic test, 68% of counselors stated they would not inform their own insurers while 28% indicated they would use an alias [30]. Uptake of counseling and testing was demonstrated in a recent HNPCC study of 446 at-risk relatives to be higher in Finland where discrimination is prohibited compared to the US [31].

Many countries and states now have legislation to counter genetic discrimination but, again, it is the patient's perception of risk that needs to be addressed during counseling. Michie et al. found that APC-gene positive patients who perceived the disease to be more threatening experienced greater anxiety [32]. This knowledge can be used during the precounseling stage to help the patient and reduce anxiety levels. Loader et al. found that acceptance of counseling for HNPCC may be correlated with parental status, increased family history of cancer, or increased social supports [33]. Pretest counseling was demonstrated to reduce fear of cancer and death in mutative-positive patients in the aforementioned Finnish study [31].

Interestingly, a minimum waiting period of two weeks is invoked for their patients as part of the decision-making process. An overview of the psychological issues which may arise and a meta-analysis of the impact of counseling illustrate common themes such as survivor guilt for unaffected relatives; parental guilt for a child; family pressure on the proband to be tested; and fear about disclosing genetic information to significant others [34, 35]. The following chapters will examine indigenous approaches to genetic counseling and testing.

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Chapter 25
Across Culture and Health Systems: Europe

Marie Luise Bisgaard

Abstract The barriers to healthcare access and genetic counseling vary from country to country. In this chapter, an European perspective to this is given through the experience of a 35 year old male name Igor John.

Keywords Across • Culture • Health • Systems • Europe 30 – Genetic • Counselling • Across • Culture • Health • Systems

25.1 Introduction and Background

Europe has natural geographic borders on three sides, namely the Arctic Ocean to the north, the Atlantic Ocean to the west and the Mediterranean Sea to the south, assuming that one includes the islands in the Mediterranean as European. The definition of the eastern border – the border between Europe and Asia – has been influenced by culture and history and is most frequently defined as the Caucasus Mountains, the Black Sea and the waterways connecting the Black Sea to the Mediterranean. Parts of Russia, Kazakhstan and Turkey fall within both Europe and Asia.

The total population of Europe is approximately 730 million. There is a tendency towards population growth in the west and a decline in the east and an overall tendency to decline. Of her 50 countries, 27 are member states of The European Union. These show high degree of mutual policy, either established or under development, forming an economic entity. In 2008 three countries became candidates for membership of The European Union. The remaining 20 countries can roughly be divided into three groups, a larger group consisting of 12 former communist countries or Soviet Union states in Eastern Europe, a group of five very small independent states scattered over south-western Europe and, lastly, three independent countries in the north-west. The European countries constitute, (see Fig. 25.1):

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Fig. 25.1 Map of European countries and border between Europe and Asia. (Made by ML Bisgaard)

The European Union	
Austria	*The candidate countries for The European Union*
Belgium	Croatia
Bulgaria	Macedonia
Cyprus	Turkey
Czech Republic	*The former communist countries or former Soviet Union states*
Denmark	Albania
Estonia	Armenia
Finland	Azerbaijan
France	Belarus
Germany	Bosnia and Herzegovina
Greece	Georgia
Hungary	Kazakhstan, European part
Ireland	Moldova
Italy	Montenegro
Latvia	Serbia
Lithuania	Russia, European part
Luxembourg	Ukraine
Malta	*The remaining independent states and countries*
Netherlands	Andorra
Poland	Iceland
Portugal	Lichtenstein
Romania	Monaco
Slovakia	Norway
Slovenia	San Marino
Spain	Switzerland
Sweden	Vatican City
United Kingdom	

25.1.1 Languages and Religions

The majority of European languages originate from six main linguistic groups, each with multiple languages and dialects. Several other languages with a different origin are also spoken. Christianity is by far the most popular religion, followed by Islam, which is particularly prominent in southeastern European countries. Many European countries have significant non-religious populations.

25.1.2 History

The culture and political systems in Europe were formed over several thousand years, with shifting focuses of power and influence. The period after the Second World War was characterised by two different tendencies. Firstly, there was an overall and ongoing movement towards integration and collaboration between the independent states. Most notable was the foundation and growth of The European Union, which started as an economic collaboration between six neighbouring states and developed into a socio-economic political unity comprising more than half of the independent European countries, which recently have come to include former communist countries. Secondly, there was a tendency towards breakaway, especially immediately after the dissolution of the Soviet Union, where several new states were founded, some of these after years of war and internal conflict in the 1990s.

25.1.3 Politics

Majority of the non-European Union member states in West Europe are very much on a par with each other and have close collaboration on most issues, both among themselves and with the European Union. European Russia, together with some of the former Soviet states and their associates, form the second largest entity or group, called the Commonwealth of Independent States (CIS) after the dissolution of the Soviet Union in the autumn of 1991. In later years, several of the 12 original member states have loosened their ties and moved towards the West [1–3].

25.2 Healthcare Systems in Europe

The World Health Organisation (WHO) gives a very broad definition of healthcare systems:

"All the activities whose primary purpose is to promote, restore or maintain health" [4]. This means that according to the WHO: "Health systems have a responsibility not just to improve people's health but to protect them against the financial cost of illness – and to treat them with dignity."

The report states the health systems accordingly have three fundamental objectives:

1. Improving the health of the population they serve
2. Responding to people's expectations
3. Providing financial protection against the costs of ill-health

The healthcare systems in Europe appear relatively homogenous when compared with other continents like Africa and South America. Yet some Intra-European differences are noticeable, particularly when comparing the countries in the former communist group in the East (Eur-B+C) with the other European countries in the West (Eur-A) as defined by WHO [5], see Fig. 25.2.

Fig. 25.2 Map of European countries according to WHO division in countries with low child and adult mortality in the west (Eur-A) and countries with higher levels of mortality in the east (Eur-B+C)

Eur-A: 27 countries with low child and adult mortality
Andorra
Austria
Belgium
Croatia
Czech Republic
Denmark
Finland

(continued)

(continued)

France
Germany
Greece
Iceland
Ireland
Israel[a]
Italy
Luxembourg
Malta
Monaco
The Netherlands

Eur-A: 27 countries with low child and adult mortality

N orway
Portugal
San Marino
Slovenia
Spain
Sweden
Switzerland
United Kingdom

Eur-B+C 26 Countries with higher levels of mortality

Albania
Armenia
Azerbaijan
Belarus
Bosnia and Herzegovina
Bulgaria
Estonia
Georgia
Hungary
Kazakhstan
Kyrgyzstan
Latvia
Lithuania
Montenegro
Poland
Republic of Moldova
Romania
Russian Federation
Serbia
Slovakia
Tajikistan[a]
TFYR Macedonia
Turkey
Turkmenistan[a]
Ukraine

[a] These states are, by geographic definition, not European

By this definition, Europe is divided into West (Eur-A) and East (Eur-B+C), where the latter group comprises almost exclusively of former communist countries and Soviet Union States, Turkey being the exception. The Czech Republic and Slovenia, are not included, as they are found in Eur-A. The following statistic data refer to this division of Europe and the underlying figures are extractions or calculations found in the WHO healthcare database for Europe [5].

To illustrate some of the differences in the European health systems, we will follow two patients afflicted with hereditary colorectal cancer (HNPCC also named Lynch syndrome) and their families' way through their healthcare systems. First, there is Igor from a former communist country in East Europe with a total population in 2005 of approximately 457 million inhabitants (Eur-B+C population). Second, there is John from a European Union member state with a total population of approximately 422 million inhabitants (Eur-A population). Because WHO includes more countries and regions, the total population (Approx. 970 million) exceeds the population found in geographic Europe (730 million).

Igor-John, who is a 35-year-old man, takes ill with severe stomach pain and he is accepted at the emergency ward, where he is diagnosed with colon obstruction. A carcinoma in cecum is diagnosed and he needs an immediate operation, but let us first look at his background.

Demographic factors: In order to find figures that can compare the burden of disease between countries, the measure disability life expectancy (DALE) has been developed.

The DALE figures from each country are extractions from the particular country's epidemiological data concerning death information and major disabling conditions. Igor's DALE is taken as the calculated average for East European countries and John's is likewise taken from European Union countries. In Eastern Europe, the average DALE was 60 in 2002, compared with 72 in Western Europe. The population life expectancies at birth are somewhat higher than DALE, but also this measure demonstrates approximately 10 years difference between the two European parts. For Igor, there has been a very slight increase in life expectancy over the last 25 years from 68 years in 1980 to 69 years in 2005, while John's life expectancy has had a steady increase from 75 to 80 in the same period.

The total fertility rate has dropped from 2.5 in 1980 to 1.6 in 2005 in Eastern Europe, and in the same period, the percentage of population aged 65 years or more has increased from 9.1 to 11.7%. The development of the corresponding figures for Western Europe has not been as distinct: the fertility rate dropped from 1.8 to 1.6 and the percentage of population aged 65 were already higher in 1980 amounting to 13.7% and increased to 16.8% in 2005.

25.2.1 HealthCare Resources

Igor-John sees his doctor and is referred to a hospital for a colonoscopy where colon cancer is diagnosed and an operation is deemed necessary. In both East and West Europe, there has been a decline in the number of graduating physicians – most steep

in Western Europe. The number was close to ten physicians graduating per 100,000 inhabitants in 2004 for both Eastern and Western Europe. The number of nurses is also comparable at approximately 30 graduating nurses per 100,000 inhabitants. There are more hospital beds available in Eastern Europe, 711 per 100,000 inhabitants compared with 577 per 100,000 in Western Europe. Over the last 25 years, there has been a tendency in both parts of Europe towards fewer beds, which could indicate more intense and rapid treatment as demonstrated by a 5- to 7-day decrease in average length of in-hospital stay over the last 25 years, down to 10–11 days in 2004. The decrease probably also reflects that restitution has been shifted out of the hospital setting.

25.2.2 Financing

The financing of the healthcare system can originate from a variety of different sources and frequently comprises a mixture of these sources. All or nearly all healthcare expenses in the former Soviet Union and the former communist countries were considered a public, governmental matter and the private households' out-of-pocket payment was almost non-existent. This has changed considerately after the change of their political system, and the private households' out-of-pocket payment now comprises more than 30% of total health expenditure, whereas in Western Europe it only amounts to a little more than half of this, namely 17% in 2004.

Charity, which along with private households' out-of pocket payments, is one of the two oldest forms of healthcare financing, plays a minor role in the financing of healthcare in modern Europe.

Taxes, optional or non-optional insurances and employer insurance or contributions are of variable importance in today's Europe. Few countries have taxes as the only public healthcare financing, but all countries use tax to finance at least part of their healthcare system for persons without other financial means. WHO has given estimates of the percentage of healthcare financing taken off by government or government-like entities, such as the Ministry of Health and social security agencies. In 2004, the estimate amounted to more than 75% in Western Europe and to around 62% in Eastern Europe.

The European Observatory on Health Care Systems [6] also take "informal payment" into account and explain that this may include gifts or direct payment before or after receiving healthcare services. It is feared that the informal payment may take the form of corruption, which undermines the official payment system. It is stated that, possibly due to insufficient research, the informal payment does not exist in Western Europe, while it is known in inpatient and outpatient care in Eastern European countries.

25.2.3 Services Provided

The hospital equipment and service that Igor encounters is somewhat different from the one that John encounters, because the total health expenditure, PPP$ (Purchasing

Power Parities in dollars) per capita according WHO estimates are approximately five times as large (2696 PPP$) in Western Europe than in Eastern Europe (526 PPP$). This figure does not only reflect a relatively smaller gross domestic product (GPD) in Eastern Europe, but can also be attributed to a lower percentage of gross domestic product being allocated to total health expenditure, in Eastern Europe (6.22%) compared to Western Europe (9.25%), according to WHO estimates for 2004.

25.2.4 Access to HealthCare Service

The healthcare system should ideally provide the optimal service to all of those who need it. Not only have economic factors (formal and in particular informal financing) compromised this ideal, but geographic distances, knowledge or educational levels and socio-cultural factors also play a role. Further, the constant progress in techniques, drugs and imaging changes the idea of optimal service from day to day. As a consequence, there is an increasing need for the development of *best practise guidelines* and the distribution of knowledge among caretakers. This rapid progress, in general terms, means that more and more cases can be diagnosed, treated and/or prevented, which all tends to be increasingly costly and to challenge the fairness in contributions. WHO have analysed the concept of fairness in health systems and says: "*Fair financing* in health systems means that the risks each household faces due to the costs of the health system are distributed according to ability to pay rather than the risk of illness" [4]. The fairness in financing is measured in UN member states by an index starting with 0 for extreme inequality and ending with 1 for perfect equality. The average index figure for those of the East European countries which existed at the time of the analysis and were members of WHO, was slightly lower than the West European figures; namely 0.91 vs. 0.97. So, all in all, it was a bit more difficult for Igor than for John to get the adequate medical attention.

25.2.5 Colorectal Cancer Incidence and 5 Year Survival

Igor-John is quite nervous about the outcome of his operation and with good reason. Although there has been a general improvement in 5-year survival of both colon and rectal cancer [7], the 5-year survival after colorectal cancer is still low: 30% in Eastern Europe and 41% for Western Europe, according to Global statistics 1999 [8]. The same source found that the incidence of colorectal cancer was 18.47 cases per 100,000 population in Eastern Europe and considerately higher in Western Europe, namely 29.01 cases per 100,000 population. This places Western Europe in a group with other high colorectal cancer regions like Australia and North America, while Eastern Europe belongs to the group of moderately high

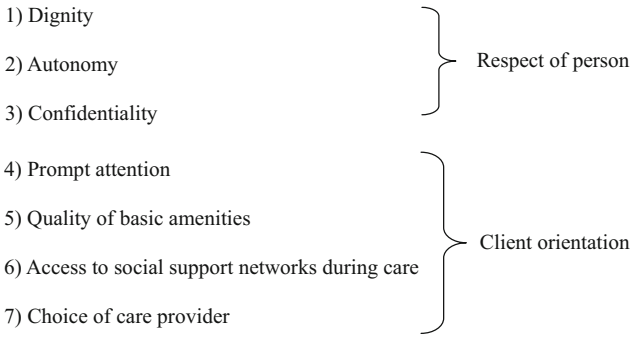
colorectal cancer incidence along with South America (Global statistics divides the world into 23 regions and Europe comprises four of these regions: Northern Europe, Southern Europe, Eastern Europe and Western Europe).

25.2.6 Performance and Overall Goal Attainment

In order to compare different countries' performance both on the level of health and the overall performance of the health systems, WHO indexed member countries according to two different systems. Performance on health estimation is based on DALE and can roughly be described as the ratio between what has been achieved and what could have been achieved at the same amounts of $PPP spent per capita. In the estimation of the overall performance of the health systems, factors such as education are also included, thus relating overall health system achievement to health system expenditure. There is a wide gap in the achieved mean index figures for overall performance estimated by WHO in 1997 between the two groups of European countries, Eastern Europe 0.693 and Western Europe 0.924, where 0 is no performance at all and 1 is a perfect performance [4].

25.2.7 Responsiveness

Luckily the operation went well for Igor-John, so he was very content with the outcome of the healthcare system. The level of responsiveness reflects to which degree the healthcare system lives up to the expectations of the consumers. Several models and methods have been developed to measure responsiveness. WHO asked key informants to score seven variables on a scale from 0 to 10, where 10 is the maximum score. The seven variables to be scored were:



The evaluation has its main emphasis on interpersonal relations and administrative smoothness and places less weight on the actual performance and outcome of the treatment.

The Eastern European countries had a mean score of 5.19 while the Western countries tended to be a bit more satisfied with their healthcare system, which received a mean score of 6.58 [4].

25.2.8 Reservations About the Statistical Material

It should be kept in mind that all the statistical material collected for comparative use is, to some extent, encumbered with uncertainty, particularly when the figures are ratios based on several estimations, as is the case for the overall performance of the health systems. This statistical material is intended to give an idea of the differences, in general, between Eastern and Western Europe. The strength of these applied statistics relies on the compiling of data from many different countries, which evens out insignificant variations.

Furthermore, it should be kept in mind that Eastern Europe has experienced enormous changes in their political and economic structures, which in some aspects – particularly those related to healthcare – has set them back to a lower level than they were before the changes. As the statistics mainly give a snapshot of the situation at a given time, they often fail to give an impression of ongoing developments.

Finally, it should be kept in mind that the variables examined might be influenced by non-transparent factors, which would vary from country to country. The sudden, and at times drastic, changes that Eastern Europe has experienced since 1991 might reflect in their evaluation of responsiveness, where the increasing out-of-pocket financing might result in greater expectations from services given.

25.2.9 Handling of HNPCC in the Health Care System

When the admission journal was taken on Igor-John prior to his operation it was picked up during anamneses that his older brother, his mother and his mother's brother have previously been operated on for colorectal cancer. The family history along with Igor-Johns young age at diagnosis with cecum cancer, give good reason to suspect hereditary colorectal cancer (HNPCC; Lynch syndrome) in his family. Questions posed would include which type of operation should be chosen, is molecular genetic analysis available, who informs family members, etc. Information concerning the handling of HNPCC in Eastern European countries is not available probably because the doctors in East European countries are to some degree still suffering from historically determined diminished contact with the western parts of the world. Very little is published or known about the handling of HNPCC in East

European countries and the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) [9] does not have members from Non-EU East European countries. The following will therefore focus on John's disease and on his family.

To get a status on the handling of HNPCC in Europe today, a questionnaire was sent to members of InSiGHT [9] where 11 experts from eight different EU countries chose to answer; the results of the questionnaire have not been published previously. In John's case, where HNPCC was suspected because of the family history, and an operation was acutely needed, the choice of operation was a subtotal colectomy and an ileorectal anastomosis.

The types of molecular genetic analysis, which subsequently were chosen, showed regional and even national differences. In some regions, all colorectal cancers were subjected to immunohistochemistry analysis (IHC), either alone or together with an analysis for microsatellite instability (MSI). In other regions or countries, it was entirely up to the clinical geneticist to whom John would be referred, to decide which molecular genetic analysis should be performed. Generally John would receive initial information about HNPCC from the operating surgeon, who subsequently would refer him to clinical genetic counselling – in most but not in all cases because in some countries surgeons with special knowledge and interest in HNPCC handles genetic counselling and in some countries clinical genetics is not established. John would be offered testing for mutation in mismatch repair (MMR) genes, unless the IHC and/or MSI contradicted this. The surgeon or clinical geneticist would then arrange that John was offered regular prophylactic examinations, comprising at least a colonoscopy every second year, while in some countries the screening interval would be 12 months and in others an examination for blood in urine would also be offered. In most countries, John's family members-at-risk would receive information about the possibilities of gene testing and/or regular prophylactic screening of colon and rectum, passed on from John personally. Only in Denmark and Finland do the national HNPCC registers contact family members-at-risk directly. In most countries, John would be prompted to inform his family members-at-risk, but because the clinical geneticist or surgeon cannot contact the family members without consent, it is unknown whether those family members who never start on prophylactic examinations have been informed and chosen not to take the offer or have simply not been informed. Studies have demonstrated that people in John's situation experience the informing of family members as a heavy burden [10].

If a germline mutation is identified in John, then all his family members-at-risk can be tested for their carrier status, and asymptomatic gene carriers will generally not subsequently experience any problems with insurance companies This is, however, not the case in the United Kingdom, where those of John's family members who are gene carriers may have problems when trying to get a life insurance. If John did not have a life insurance before his operation, he could face difficulties in getting one in the first years after his operation in most countries.

Shortly after John's operation, he has informed his 22-year-old sister Mary about the diagnosis of HNPCC and she chooses to see her doctor right away because she has just realised that she is pregnant. In most countries, Mary would be referred to genetic

counselling, where they would suggest that she should wait with further action until after delivery. After delivery, she would be offered a gene test and/or regular prophylactic examinations. In most countries, examinations would include gynaecological examination for endometrial cancer and in some regions for ovarian cancer.

In the vast majority of European Union member states, John and Mary would have no or very little out-of-pocket payments in connexion with their treatment and surveillance examinations. The patients have free access to a general practitioner who functions as the gatekeeper and refers the patients to specialists or hospitals as and when needed. Private hospitals play a minor role in today's Western European countries.

John and Mary will be asked to consent to registration in their countries' or regions' HNPCC registers, where clinical and genetic information is kept for both surveillance and research purposes.

25.3 Summary

The healthcare systems in Europe are broadly speaking quite comparable, but there are differences, particularly between countries in the East and the West. Eastern European countries have experienced major changes after the dissolution of the Soviet Union and Europe as such is progressing towards greater and greater integration and collaboration between the independent states. In Western Europe, the HNPCC is afforded serious attention, and molecular diagnosis and surveillance programmes for family members-at-risk are instituted. With minor exceptions, patients are treated and followed as suggested by the Mallorca group [11].

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Chapter 26
Across Culture and Health Systems: Asia (Hong Kong)

Judy Wai-chu Ho, Samuel Mun-yin Ho, and Annie Tsz-wai Chu

Abstract This chapter will discuss the approach given to patients and their families with hereditary colorectal cancer in Hong Kong. The workflow utilized by the Hereditary Gastrointestinal Cancer Registry as well as social and cultural issues unique to Hong Kong will be presented. Genetic counseling, testing, support, and economics of these will also be presented.

Keywords Hereditary colorectal cancer • Chinese • Registry • Genetic testing • Counseling • Psychosocial support

26.1 Introduction: Colorectal Cancer in Hong Kong

In Hong Kong, colorectal cancer (CRC) is an increasing healthcare burden. According to the latest statistics, CRC is the second commonest cancer and the second cancer killer with 3,582 new cases and 1,538 death cases in 2004 [1]. There has been a progressive increase in the rate of CRC in the past decades, and by 2010, CRC will surpass lung cancer to become the commonest cancer in Hong Kong.

Comparing two time periods of 1983–1993 vs. 1994–2003, the main increase in CRC incidence can be attributable to classical, late-onset cancer (age above 50 years old) for which environmental factors have been thought to be the main cause (see Figs. 26.1 and 26.2). However, Hong Kong has a much higher incidence of CRC in the young age group (under 40 years old) than other countries [2] and a significant proportion of these young CRC have been shown to be due to hereditary predisposition [3].

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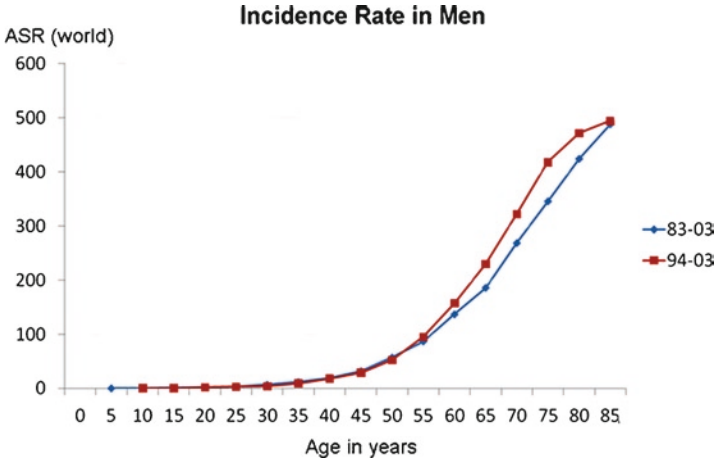


Fig. 26.1 Age-standardized incidence rate of colorectal cancer in men from Hong Kong over the two time periods of 1983–1993 and 1994–2003

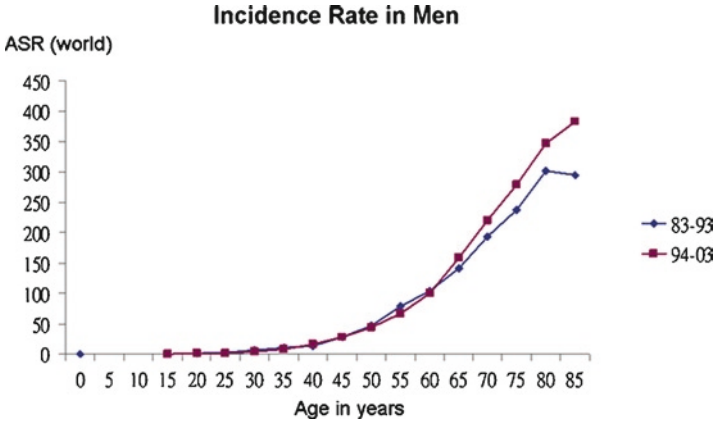


Fig. 26.2 Age-standardized incidence rate of colorectal cancer in women from Hong Kong over the two time periods of 1983–1993 and 1994–2003

26.2 The Hereditary Gastrointestinal Cancer Registry

To fill the gap in our knowledge regarding hereditary colorectal cancer (HCRC) in Hong Kong and to meet the service needs of susceptible families, the Hereditary Gastrointestinal Cancer Registry (the Registry) was established in 1995 by a group of medical specialists working at Queen Mary Hospital which is one of the teaching hospitals in Hong Kong. The mission of the Registry is "to prevent CRC in high-risk families through early detection, timely treatment, education and ongoing research."

Table 26.1 Recruitment criteria of the hereditary gastrointestinal cancer Registry

1. Families affected by histologically proven Familial Adenomatous Polyposis (FAP) or other polyposis syndrome. This includes index patients and at-risk first-degree relatives above the age of 12 years.
2. Families affected by Hereditary Non-Polyposis Colorectal Cancer (HNPCC) satisfying the Amsterdam Criteria, the modified Amsterdam Criteria and/or with proven germline mismatch repair gene mutations. This includes index patients and at-risk first-degree relatives above the age of 25 years.
3. Suspected HNPCC families satisfying one of the following criteria:
   - An individual has histologically proven colorectal cancer diagnosed before the age of 45 years.
   - An individual has two HNPCC-related cancers, including synchronous and metachronous colorectal cancer or associated extra-colonic cancers.
   - An individual has histologically proven colorectal cancer and a first-degree relative has histologically proven colorectal cancer or HNPCC-related extra-colonic cancers. At least one of these cancers is diagnosed before the age of 45 years.

   For those families, we recruit both index patients and at-risk first-degree relatives above an age that is 5 years younger than the youngest age of cancer diagnosis in the family
   Extra-colonic cancers include: cancers of the stomach, small bowel, uterus, ovary, brain, and transitional cell carcinoma of the urological tract

The Registry's recruitment criteria are shown in Table 26.1. It accepts referral by medical or paramedical professionals as well as self-referral by the families themselves over the whole territory of Hong Kong. By December 2006, the Registry has received more than 2,600 referrals with recruitment of more than 640 families. Referral sources are: 76.6% public hospital, 18.0% self-referral and 5.4% private specialists [4].

The work of the Registry can be summarized into four main areas which include clinical service, education cum training, research and international collaboration. Table 26.2 summarizes key work of the Registry in each of the above-mentioned areas.

The Registry team consists of:

1. A clinician/surgeon who is responsible for the administration of the Registry and supervises risk assessment, clinical management, and counseling
2. A Registry coordinator who serves as a liaison between recruits and medical specialists. She is also responsible for the day-to-day running of the Registry
3. A scientist/pathologist who supervises molecular genetic analysis
4. A clinical psychologist who is responsible for psychosocial support of recruits
5. A clerical assistant who provides clerical support and assistance to the coordinator

There are also affiliated members from other hospitals or institutions including surgeon liaisons to provide assistance in family recruitment, clinical surveillance, and management; pathologist liaisons to assist in tumor tissue tracing and case identification; and psychosocial liaisons to support psychosocial research and service.

Table 26.3 outlines the workflow adopted by the Registry in the work-up of newly referred families.

Table 26.2 Major areas of work of the Hereditary Gastrointestinal Cancer Registry

Clinical service
1. Medical record compilation and database management for recruited families.
2. Genetic diagnosis and predictive genetic testing – for suitable families, the Registry arranges genetic analysis for Familial Adenomatous Polyposis (FAP), Hereditary Non-Polyposis Colorectal Cancer (HNPCC) and Peutz–Jeghers Syndrome (PJS).
3. For mutated gene carriers and at-risk family members with uninformative genetic status, regular clinical surveillance will be arranged in regional hospitals. If required, individuals will be referred for surgical treatment in specialist units.
Education and psychosocial support
1. Education of recruits through educational booklets, talks, seminars, half-yearly newsletter, and website.
2. Education of medical professionals through educational talks and presentations in local medical conferences to ensure proper referral.
3. Psychosocial support to recruited members.
Research
Clinical, psychosocial, and molecular genetic researches are being conducted to improve our understanding and management of hereditary colorectal cancer.
International collaboration
Through international meeting and collaborative research effort, the Registry aims to facilitate exchange of medical information and multicenter studies.

In the remaining sections, we shall focus on genetic counseling, psychosocial research, and psychosocial service of the Registry. We shall also highlight some of the important issues relating to these areas in Hong Kong.

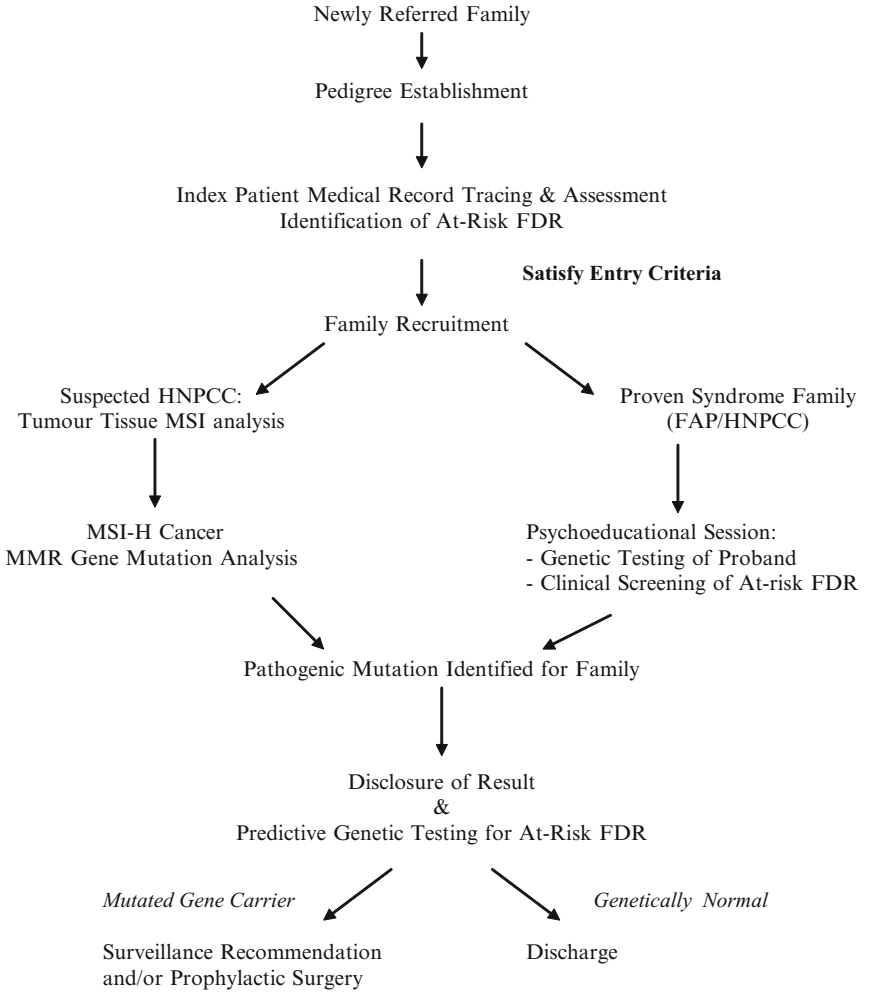
26.3 Psychosocial Impact of Hereditary Colorectal Cancer in Chinese

During the initial phase of managing local syndrome families, Registry staff encountered different responses of recruits to their hereditary predisposition. For some of them, the psychosocial impact of HCRC had significant and adverse effect. However, there was little information in the literature regarding psychosocial adjustment to hereditary cancer in Chinese; none was found for HCRC until 2001. With the help of experts from The University of Hong Kong, the Registry pioneered on psychosocial research in Chinese HCRC families. The following two subsections summarize some of the findings of our research.

26.3.1 Influence of Chinese Culture

Chinese culture has important influence on our recruits' adjustment to HCRC. All along, the Chinese consider cancer as a curse. The inheritance of cancer from one

Table 26.3 Workflow of the Registry for newly referred families



FDR first-degree relative, *HNPCC* hereditary non-polyposis colorectal cancer, *FAP* familial adenomatous polyposis, *MSI* microsatellite instability, *MSI-H* high level of microsatellite instability, *MMR* mismatch repair

generation to another has been regarded as a family curse. During qualitative interviews with four syndrome patients diagnosed for more than 5 years [5], we detected a strong influence of this concept in the initial phase of these individuals' illness experience. For example, HCRC was regarded by one recruit as a curse due to improper burial of the ancestors.

However, with time, these recruits were able to dispel such "family curse" and turn the curse into a more welcomed "blessing" with the help of Buddhist philosophical ideas including: accepting and finding meaning in suffering; accepting the

unpredictability of life; embracing the virtue of perseverance; being virtuous and helping others; letting go; and growth through pain. Using these philosophical ideas, our recruits were transformed and were able to change their views on life and death toward more positive ways.

Although HCRC is a family issue, there may be a tendency for our recruits to avoid sharing their emotional turmoil with their family members, not because they think that the other family members are unsupportive, but because they do not want to impose burdens and suffering on their loved ones [6]. The opportunity to share one's emotional reactions toward HCRC genetic screening without having to worry about burdening the other family members is especially important among the Chinese.

Moreover, Chinese culture plays an important role in decisions relating to genetic testing. In another study [7], we found that our Chinese recruits were relationally and interdependently orientated in their decisional consideration process; that is, our recruits were concerned about the well-being and reactions of their significant others even more than their own well-being when they decided whether to receive genetic testing or not. This finding has a significant impact on our subsequent pre-testing genetic counseling strategy.

26.3.2 Genetic Testing

Genetic testing for HCRC has potential benefits and disadvantages for the recipients. Existing literature showed that up to 43% of adults who were tested positive for HCRC were clinically anxious after receiving the genetic testing results [8]. Psychological distress among gene carriers is understandable because they have to face the uncertainty regarding self-onset of cancer, the possibility of passing the mutated gene to their children as well as the possibility of genetic discrimination [9–14]. Although non-carriers may experience relief from their test results, they may also exhibit negative psychological reactions including feeling of survivor guilt, disbelief about their test results due to preconception of susceptibility, and repercussions on family relationships [15]. Similar to the experience of other countries [16], not all members of our recruited families are willing to participate in HCRC genetic testing or to learn about their test results.

In our qualitative interviews [5], our subjects expressed anticipatory anxiety before disclosure of genetic testing results. They also expressed their concern regarding informing relatives about their genetic testing results. It was because they regarded hereditary cancer as a family curse and hence disclosure of this condition equated to bringing bad news to the family members.

In our study on decisional consideration [7], we found that subjects with higher perceived risk of cancer tended to emphasize more on the negative consequence of learning their test results. We suggested that psychological counseling would help these individuals to cope with their anticipatory anxiety and the subsequent distress if proven to be gene carriers. Besides, subjects with higher depression level focused more on the harmful consequence of sharing their test results with relatives.

Therefore, psychological services to reduce distress among individuals going through genetic testing might be beneficial. Furthermore, we found that subjects who believed that cancer was due to bad luck or fate (uncontrollable factors) rather than personal factors (such as stress) tended to focus more on the positive aspects of sharing their results with relatives.

26.4 Psychosocial Program

On the basis of the findings of our research, the Registry has identified the following areas of psychosocial support for our recruits:

1. Psychoeducational program for genetic testing
2. Psychosocial program for mutual support
3. Intervention based on the theory of positive psychology

26.4.1 Psychoeducational Program for Genetic Testing

Our previous findings showed that our recruits tended to focus on the negative aspects of HCRC genetic testing. This may be partly due to a lack of knowledge about HCRC and its genetic testing among Hong Kong people. Existing literature also shows that cancer knowledge is an important factor affecting adjustment to HCRC [13]. Therefore, increasing the subjects' knowledge is a common strategy to enhance adjustment [17].

26.4.1.1 Educational Material

Over the years, a protocol for genetic counseling has been established in the Registry. Based on the protocol, a manual titled "Hope-Based Intervention Study, Psycho-Educational Component: a Framework" was published in January 2006 [18]. Figure 26.3 shows the front page of our manual and Table 26.4 gives a brief outline of its content.

In the manual, separate subsections are written for FAP and HNPCC regarding their respective clinical features, genetic features, clinical management and surveillance protocol. Subsections with revised risk assessment and revised management guidelines are also written for mutated gene carriers as well as genetically normal first-degree relatives.

Apart from serving as a document for our psychosocial intervention program, the manual is also a useful resource to standardize the psycho-educational process of HCRC genetic testing for quality assurance. Furthermore, the manual can be used as an educational tool for training future genetic counselors in Hong Kong.

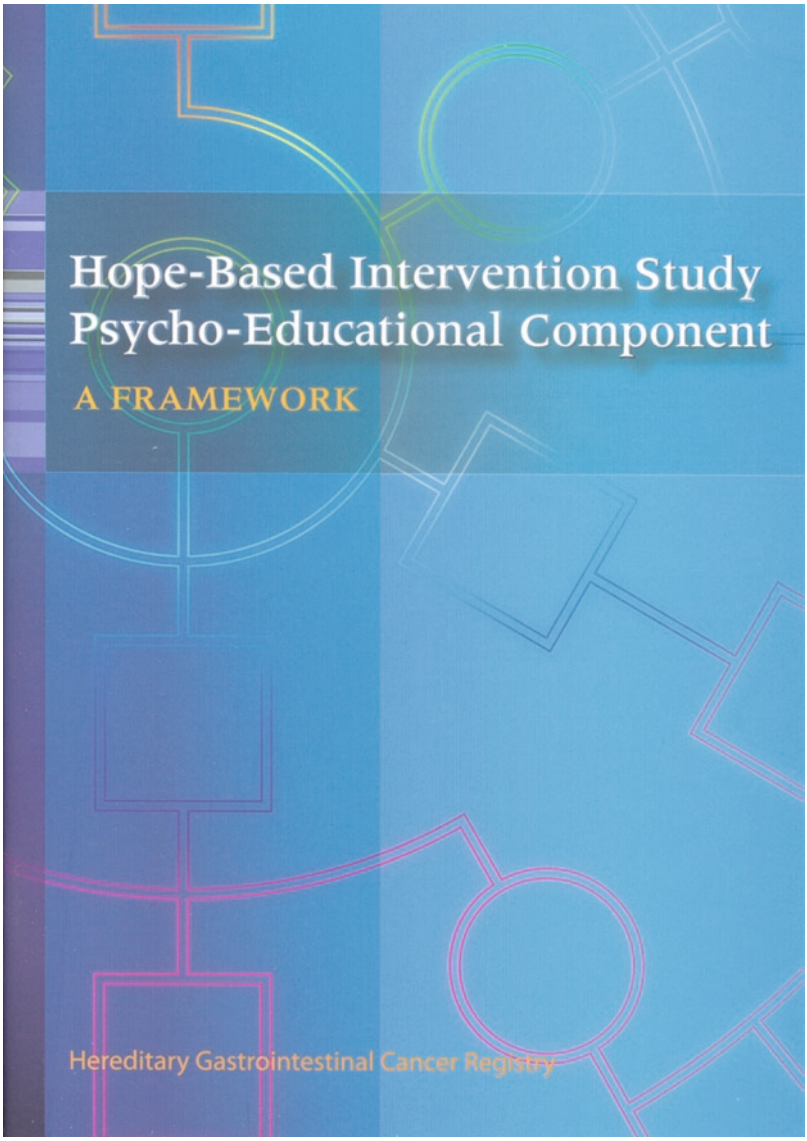


Fig. 26.3 Front page of the psychoeducational manual published by the Registry

26.4.1.2 Genetic Counseling

For newly referred individuals eligible for genetic testing, the Registry will provide genetic counseling on an individual basis before genetic testing and upon disclosure of genetic testing results.

Table 26.4 Content outline of the psychoeducational manual published by the Registry

Background on hereditary colorectal cancer and the Registry
Referral and recruitment criteria of the Registry
Purpose of the manual
Pre-counseling risk assessment – pedigree establishment and medical record assessment
Pre-testing counseling session
Cooling period and informed consent for genetic testing
Counseling session for disclosure of genetic testing result – revised risk assessment and clinical management recommendation
Post-disclosure follow-up
Conclusion

26.4.1.3 Pre-testing Genetic Counseling

At the beginning of the counseling session, the counselor will explain the purpose of the session and record any update of medical histories in the family concerned. Education will be provided for the clinical and genetic features of the suspected syndrome. This will be followed by a discussion on screening including the purpose of screening and our recommendation regarding clinical and genetic screening. The use of genetic testing and its pros and cons will also be discussed. Reassurance would be given regarding confidentiality of genetic testing information. Possible psychological reaction relating to genetic testing will also be mentioned briefly. At the end of the session, a question and answer time will be provided to allow the recruit to clarify any issues. A cooling period will be allowed before an informed decision is made by the recruit regarding genetic testing.

26.4.1.4 Group Psycho-Education for Extended Family

For extended family of large size, a dedicated group session has been used as an efficient and time-saving way to provide education and counseling for family members before genetic testing. The whole family would be invited to attend the group session conducted over a weekend. Efforts are put into ensuring that at-risk first-degree relatives (with spouses if married) as identified from the pedigree will be able to participate. It is particularly important to include at-risk family members at or above the appropriate age for screening.

The educational content will be similar to that of an individual's pre-testing genetic counseling session. Audiovisual aids are often used to maintain attention and to improve understanding of the information discussed. A question and answer period will also be given at the end to ensure understanding and to allow the Registry staff to answer specific questions related to individual family members. If required, further follow-up sessions for individuals would be arranged before an informed decision can be made regarding screening and genetic testing.

26.4.1.5 Procedure for Disclosure of Genetic Testing Result

Upon availability of genetic testing result, the Registry will inform the recruit by mail inviting the recruit to make an appointment for result disclosure. If agreeable, the recruit will contact the Registry for an appointment of post-testing genetic counseling.

26.4.1.6 Post-testing Genetic Counseling

The post-testing genetic counseling will be conducted by a panel consisting of the clinician, the clinical psychologist, and the coordinator. At the beginning of the session, the counselor will explain the purpose of the visit and update any family medical information if necessary. The result of genetic testing will then be disclosed with explanation of its implication. Education will be given on the clinical and genetic features of the syndrome. Based on the genetic testing result, a discussion will be held on the revised risk estimation and the revised clinical recommendation for the recruit.

For proven mutated gene carriers, discussion will be held on the importance of secondary prevention for both colonic and extracolonic cancers. For genetically normal individuals, the counselor has to ensure proper understanding of this individual about a normal test result. Discussion will then be expanded on family management, confidentiality issue regarding testing results and social issues including marriage, procreation, and social discrimination. Recruit will be made aware of the possible immediate and delayed psychological reactions related to either a positive or negative genetic testing result so that further support can be offered when required. Our two patient support groups (one for FAP and another for HNPCC) will be introduced and recruit will be encouraged to attend functions held by the support groups. Finally, a question and answer time will be reserved to allow recruit to clarify any issues concerned.

During the session, Registry staff will detect untoward reaction of the recruit to the testing result and will identify individuals who may require further follow-up care by the clinical psychologist.

26.4.1.7 Post-disclosure Follow-Up

The Registry coordinator or the clinical psychologist will conduct a post-disclosure telephone follow-up about 1 month after result disclosure to clarify issues and to detect the need of the recruit for further support and counseling.

26.4.2 Psychosocial Support Program

26.4.2.1 Patient Support Groups

Two patient support groups, one for FAP and another for HNPCC, have been established to provide regular education and psychosocial support to individuals with the respective syndromes.

Regular educational talks and seminars would be held to inform members on any new development regarding the clinical management of the respective syndromes. Sharing session would be conducted to allow fellow members to share ways on coping and living with their syndrome. Special sessions including art therapy, relaxation exercise and stress-management workshop have been held to enhance psychological support.

In collaboration with the Family Institute of The University of Hong Kong, family sessions have been conducted in which family members are encouraged to share among themselves ways of coping with the syndrome and to discuss on issues affecting family relationship due to the syndrome. These sessions have been proven useful in enhancing communication among syndrome family members.

Social gatherings are also held by the patient groups at regular intervals so that fellow members can get to know each other better in a social environment.

26.4.2.2 Ad Hoc Peer and Professional Psychological Support

Prior to prophylactic surgery for FAP, the Registry will arrange experience sharing by a fellow FAP individual. The patient will be matched with a fellow-patient of the same sex, of similar age at prophylactic surgery and who had undergone the same type of preventive surgery. Through telephone conversation and hospital visit, the fellow-patient will provide support and trouble-shooting for the said patient in the perioperative period so as to maximize adjustment and to reduce untoward experience during the prophylactic surgical procedure. Such an arrangement has fostered many friendships in the past. For teenagers undergoing surgery, the Registry will also arrange experience sharing and support by fellow-parents on ways to look after their offspring in the perioperative period.

For recruits requiring in-hospital treatment, the Registry will arrange hospital visits by volunteering fellow-syndrome patients to provide support. Our clinical psychologist has used psychological means to reduce postoperative pain in teenagers undergoing prophylactic colectomy. Psychosocial support is important when recruits face ad hoc life events including pregnancy, death in the family, and new cancer diagnosis in the family. During such situations, the Registry would arrange additional counseling sessions to deal with the medical and social issues involved and to provide psychological support when necessary.

The twice yearly newsletter published by the Registry serves as a forum for education of our recruits and the medical profession regarding HCRC. Recruits also submit articles and poems regularly to be posted on the newsletter to share their experience of living with their condition.

26.4.3 Intervention Based on the Theory of Positive Psychology: Future Direction of Psychological Service

In recent years, the Registry is moving from remedial psychological support toward prophylactic psychological services to improve resilience among the recruits. The

cognitive theory of hope proposed by Snyder et al. [19, 20] in helping people to cope with stressors has attracted our attention.

According to Snyder's model, hope has two interrelated cognitive components: agency and pathway. Agency refers to an individual's motivation to meet the desired goals, while pathway refers to an individual's ability to produce routes for attaining these goals [21]. This model proposes that, when confronted with negative surprise events such as a positive genetic testing results, the high hope individuals will only be distressed temporarily but will bounce back full of energy and ideas of achieving their life goals [22].

Research shows that hope is a significant predictor of psychological well-being among healthy people [23] as well as individuals under health threats such as spinal cord injury [24] and cancer [25–28]. Our latest study showed that hope was a significant predictor of depression, anxiety, and positive emotion among individuals either awaiting or being tested positive in HCRC genetic testing; and the relationship was independent of their information seeking styles and colorectal cancer knowledge [29].

We believe that increasing hope before HCRC genetic testing may increase resilience among the recruits. We have developed a hope-based intervention manual specially designed for individuals undergoing HCRC genetic testing. Our pilot study (completed in July 2006) showed that all participants were able to acquire the hope concepts and theories after six sessions of group psychotherapy. The participants also demonstrated different extent of progress in terms of mood, acceptance of illness, problem-solving skills, social functioning, and ability to derive meaningful life goals. The group was well-accepted by the participants who found the intervention beneficial and would recommend the group therapy to other recruits. We are currently conducting an outcome study to test the efficacy of such hope-based intervention.

26.5 Issues Relating to HCRC in Hong Kong

26.5.1 Government Involvement in the Management of HCRC

In 2004, the Hospital Authority of Hong Kong recognized the work of the Registry by granting the Registry team an "Outstanding Teams Award of the Hospital Authority." In December 2004, the Hong Kong Government published a report commissioned by the Cancer Expert Working Group on cancer prevention and screening in Hong Kong [30]. Although the report considered that there had been insufficient evidence to recommend routine colorectal cancer screening for the general population in Hong Kong, it recommended clinical screening for mutated gene carriers of FAP and HNPCC using the clinical surveillance protocols advocated by the Registry.

Despite the recognition of both the government and the Hospital Authority of the existence of the Registry and its voluntary work, there has been, to date, no admin-

istrative or financial support offered by either party to the Registry or to any other service on HCRC syndromes in Hong Kong. Since its inception in 1995, the Registry has been obtaining its financial support through various research grants, individual donations, and corporate donations. Large-scale fundraising activities have been held since 2006 in order to sustain and expand the Registry's work.

26.5.2 Economics of Genetic Testing

Although the report of the Cancer Expert Working Group has indirectly recognized that genetic testing has been performed in Hong Kong and genetic testing plays a role in the management of HCRC syndromes, the report has not mentioned or recommended who should arrange or finance the said genetic testing. Genetic testing for hereditary cancers has not been included as a service provided by the public health care system. Funding for genetic testing of HCRC syndromes has all along been provided by the Registry. During counseling for genetic testing, the cost of genetic testing will be mentioned. Recruits are encouraged to consider donating the said amount to the Registry on a voluntary basis to ensure continuation of our genetic testing service.

26.5.3 Training of Cancer Genetic Counselor

In Hong Kong, there is no certified cancer genetic counselor. Since the inception of the Registry, cancer genetic counseling has been provided by the Registry clinician. Since her appointment, the Registry clinical psychologist has received training and has assisted the clinician in genetic counseling. There is a pressing need to develop a formal training program for cancer genetic counseling in Hong Kong because of the increasing use of genetic testing for various hereditary cancer conditions. With its experience accumulated in the past decade, the Registry is in a prime position to spearhead the development of such a program in collaboration with medical, social, and psychological experts from the universities.

26.5.4 Legislation Against Genetic Discrimination

Although cancer genetic testing has been conducted in Hong Kong for over a decade, there has been no legislation to safeguard against genetic discrimination. Proven mutation carriers recruited by the Registry have experienced difficulty in obtaining life and medical insurance coverage. It has been a usual practice for our recruits to take their own annual leave in order to attend counseling and clinical surveillance. Often, employers and friends of our recruits are unaware of their genetic condition.

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Chapter 27
Across Culture and Health Systems: Asia (Japan)

Takeo Iwama

Abstract The outline of Japanese health system is discussed in relation to treatment of hereditary colorectal cancer. In the discussion, continuous support for patients and family members with hereditary colorectal cancer (familial adenomatous polyposis) is emphasized. It includes genetic counseling for hereditary colorectal cancer, and the activities of self-help groups. To achieve the lifetime well-being of patients with hereditary colorectal cancer, it will be necessary to know how the patients were treated and their results. We show the recent status of FAP in Japan.

Keywords Health insurance • Medical expenditure • Genetic counseling • Hereditary colorectal cancer • Ileal J-pouch anal anastomosis • Family history • Next generations • Family studies • Registry

27.1 Health Insurance System of Japan

Most Japanese people are expected to belong to one of two major health insurance systems (http://www.mhlw.go.jp/english/wp/wp-hw2/part2/p1.pdf)[1]: the Health Insurance Organization for Employee (society-managed, 31% or the government-managed, 28%) and the National Health Insurance for the other persons, 40%. Others are Seamen's Insurance and insurance by Mutual Aid Associations. As for medical care, there are no substantial differences between them. We also have the health care service for the elderly. They pay insurance premiums every month in proportion to their income. Half of the premium is compensated by companies, local governments or the nation to which they directly belong. Since the establishment of the universal medical care insurance system

[1]White paper and reports from Ministry of Health, Labor and Welfare (of Japan), http://www.mhlw.go.jp/english/wp/index.html

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in 1961, patients have had the freedom of selecting and accessing to any medical institution they like (http://www.mhlw.go.jp/english/wp/wp-hw/vol1/p1c3s1.html). All citizens have been granted to receive almost even quality medical care through the support of the medical insurance provision. Specific groups of people such as infants and people with specific diseases can receive a medical aide from local governments. Special supply such as personal room, and expensive medical instruments, medicines, and procedures that are out of the cover of the public health insurance need to be paid personally or by personal medical insurance companies. As for hereditary colorectal cancer, micro-satellite instability test for patients with a substantial possibility of hereditary non-polyposis colorectal cancer (HNPCC) has recently come within the cover of the medical care insurance. Other genetic tests for hereditary colorectal tumors are out of insurance coverage.

In these 10 years, our medical expenditure is increasing, and the imbalance between the medical expenditures and the income of the insurance premium has been widening. This imbalance is caused by the rapid increase in the rate of the aging population, introduction of high cost medical technology, and drastic changes in socio-economic situations and our employment market. At the beginning of our health insurance system, any employees could receive the regular medical care for nothing, but now they, as well as its family members, have to pay 30% of their medical expenditure. However, if the expenditure of their medical care exceeds a limited amount of money, the exceeding amount will be returned later. The Japanese health care system has been one of the causes that have kept their life expectancy being the top of the world; 78.5 years for male and 85.5 years for female in 2007. The government of Japan has passed several legislations to cut the medical care expenditure by 3% on average, while the demands for accountability of hospitals for the safety of patients, enough supply of medical information, and comfortable circumstances are soaring. Cutting medical expenditure policy has undermined the Japanese medical care environments very much. Many small hospitals that have been very convenient for people living in rural villages received more damage than the big medical institutes in urban areas. Some of the provincial hospitals quit or merged with others due to unbearable financial deficits. It is the purpose of the government to reduce the total number of hospital beds in Japan to cut the medical expenditure. It says that the policy is to make medical care more rational, cost-effective, and competitive for the nation.

27.2 Genetic Counseling in General

We have several medical societies to study genetic conditions and for genetic counselors; The Japanese Society for Genetic Counseling (since 1977), The Japan Society of Human Genetics (since 1956), Japanese Society for Familial Tumors (since 1994). These societies offer their training courses for genetic counselors. Genetic counseling in Japan originated from the counseling service for parents who had a child or children with congenital abnormalities. Genetic counseling has been offered by doctors who have majored in human genetics, gynecology, and pediatrics. Their major concerns were to determine the mode of inheritance, carrier detection, to calculate the

risk of recurrence to have a baby with the same condition, to inform the parents of appropriate information, and to support the parents to cope with the situation. The counseling has been rather transient until the birth of the baby. Medical care followed after this kind of counseling. With the general understanding of the natural history of hereditary colorectal cancer [1], the need for continuous genetic counseling for hereditary colorectal cancer is basically differnt from the traditional way of genetic counceling. Genetic counseling for patients with hereditary colorectal cancer is rather to coordinate patients with specialists and medico-social resources during their lifetime and generations. Genetic testing in Japan is offered under the "Guidelines for Genetic Testing (2003)". We can see this guide line on the web (http://jshg.jp/pdf/10academies_e.pdf). It will be more useful to refer to "Review of Ethical Issues in Medical Genetics", by World Health Organization, 2003 [2].

27.3 Genetic Counseling for Hereditary Colorectal Cancer

Interest in familial adenomatous polyposis (FAP) and Lynch syndrome or HNPCC in Japan has rapidly increased since the foundation of Polyposis Center in the Tokyo Medical and Dental University by Joji Utsunomiya in 1975 [3]. In 1980, he and his colleagues developed an ileal J-pouch anal anastomosis to preserve the anal function after excision of total colon and rectum [4]. It is now one of the standard surgical procedures for both ulcerative colitis and FAP. From a little before 1990, responsible genes of gastrointestinal hereditary cancer have been revealed one after another starting from the finding of *P53* and *APC*. The need for genetic counseling started to be recognized in Japan from this period.

At the early stage of the establishment of the Polyposis Center, we realized that it is essential to know the precise family history of patients with FAP or Lynch syndrome (HNPCC) to manage them properly. We were able to access the national family registry to determine the precise family history and causes of death of such patients with authorized permission, which is limited to a specific medical investigation. The information was provided to original doctors of the patients. The doctors used the information to make patients realize their condition and make them aware of the risk to their family members. This investigation method, including the nationwide registry, was ceased in around 1987 under the general regulation on privacy. In this approach, we realized that a surgeon could not persuade family members into having early examination, or afford to pay them continuous attention and offer them counseling without a coordinator or counselor who has good knowledge of this field. At the Polyposis Center, in an early stage, we employed social workers for patients to discuss their difficulties.

Genetic counseling for hereditary cancer should be offered to patients as well as to their family members for their life time, if they have the condition inherited, and the counseling should be continued over to the next generations whenever they need it. Genetic counseling should be continuous, and it should include family members of the patients with continuous renewal of their pedigrees because genetic counseling is based on family studies [5].

27.4 Genetic Counseling as a Medical Professional or Specialty in Japan

Genetic counseling is not established as a medical specialty in Japan because genetic counseling services are not considered as an independent medical service and are not covered by medical insurance. They have been offered as a part of the general health care systems by doctors interested in studying hereditary colorectal cancer or by social workers in the area of their duty. In a few limited hospitals, genetic counseling is offered by a genetic counselor. Although genetic counseling courses have been established in several colleges, the market for the graduates is to be developed in major hospitals and cancer centers. Genetic counseling by a specialist has been usually offered for research activities or for an educational purpose in these colleges.

A patient with hereditary colorectal tumor is lucky if there is a genetic counselor or a coordinator at the hospital. Many hospitals do not provide a genetic counselor. With the recent amazing advances in genetic and molecular biological technologies, the needs for genetic counseling may increase in many medical situations as well as for patients with hereditary colorectal cancer.

27.5 Bases for Genetic Counseling for Hereditary Colorectal Cancer

Besides general counseling techniques and genetic knowledge on the hereditary colorectal cancer, genetic counseling for hereditary colorectal cancer is based on three essential and indispensable procedures. They are (1) family studies or family history recording, (2) registry of the families [1, 5], and (3) continuous concern for patients and their family members over generations.

Family studies: our first step is to hear the complaints of patients carefully, and we ask the history of their family members. Genetic counseling starts with noting down the family history. Krush describes very high grade methods of family studies "Family Studies of Genetic Disorders by Krush AJ and Evans KA" [4]. Some of them are very useful when we take family history for genetic counseling of hereditary colorectal cancer. Careful history taking may reveal a specific condition of the family that may be a new disease. Sometimes, we have to access the original medical documents of the family members. It is a good idea to have a key person in the family to gain access to the family members. Pedigrees must be kept for a long time and it will be renewed from time to time. The genetic counselor is partly responsible to keep the working pedigree.

Registry: A genetic change in a person may appear as an epidemiological phenomenon in the community. Registry of hereditary colorectal cancer is essential for genetic counseling for hereditary colorectal cancer because it supplies basic knowledge for care of patients with such conditions. In Japan, there is no authorized and perpetual registry system for hereditary tumors. A registry office needs at least a team leader who directs the registry, a coordinator or a genetic

counselor, and a register or a family investigator [4]. Such projects cannot make new achievements every year. Most government administrators prefer to invest their budget in projects that are in vogue or in subjects that are expected to give clear and immediate results. One problem for the registry of hereditary colorectal cancer is that the general opinion in Japan is that the registry of hereditary colorectal cancer will need to get an approval from the institutional review board (IRB) of each hospital as well as to get informed consent from patients with hereditary colorectal cancer. There are ~5,000 hospitals that have 100 beds or more in Japan, and many of them do not have an IRB. Usually, a doctor is glad to explain the importance of the registry to patients and get their consent for their personal data to be registered, but he/she will not dare ask the hospital to establish and open the review board to discuss on the registry of a rare case. We have been privately managing a registry system for FAP. We presented here a clinical overview of FAP for genetic counseling for Japanese patients with FAP (Tables 27.1–27.5 and Figs. 27.1–27.5). I hope these are useful for patients of FAP, doctors, genetic counselors, or coordinators for patients with hereditary colorectal cancer.

Table 27.1 Surgical procedures and their background

	Resection of total colon and rectum	Rectum-preserving surgery	Partial resection or diminutive surgery
Number of patients	527	439	208
Male:Female	307:220	235:204	109:99
Mean age at surgery: mean ± SD years	33.6 ± 11.3	33.9 ± 11.7	39.3 ± 142[a]
Cases with advanced colorectal cancer (%)[b]	258 (490)	128 (292)	166 (798)

[a]This group was older than other groups (Turkey–Kremmer test, 0.05)
[b]Cancer in the stage ≥ T2: three groups showed significant difference from other groups (qui-square test $p < 0.001$)

Table 27.2 Causes of death of FAP: reported during 1990–2000

	Male	Female	Total	(%)
Colorectal cancer, polyposis, ileus	23	20	43	65.2
Desmoids	1	4	5	7.6
Gastric cancer	1	1	2	3.0
Duodenal or periampullary cancer	4	0	4	6.1
Pancreatic cancer	0	1	1	1.5
Small intestinal cancer	1	0	1	1.5
Pulmonary cancer	2	1	3	4.5
Uterine cancer	0	1	1	1.5
Esophageal cancer	0	1	1	1.5
Thyroid cancer	0	1	1	1.5
Brain attack	1	0	1	1.5
Heart attack	2	0	2	3.0
Other diseases	1	0	1	1.5
Total	36	30	66	100.0
Age at death (mean ± SD)	47.3 ± 13.5	43.1 ± 15.8	45.4 ± 14.6	

Table 27.3 Colorectal cancer specific postoperative survival rate

Postoperative months	Without cancer	With cancer (stage ≥ T2)
60	0.94 ± 0.01	0.51 ± 0.02
120	0.86 ± 0.02	0.43 ± 0.02
180	0.85 ± 0.02	0.37 ± 0.02

Table 27.4 Postoperative risk of desmoid tum or in cases registered since Jaunary 1990 (know and reported cases)

	Male	Female
Total cases	200	176
Postoperative desmoid	10	20

Table 27.5 Risk of rectal cancer after rectum-preserving surgery in 332 cases with FAP (mean age at the rectum-preserving surgery was 34 years). Iwama T. Dis Colon Rectum. 1994;37:1024–1026

Postoperative years	Cancer risk	95% Confidence interval (%)
5	4.0	
10	12.8	11.1–14.5
15	24.2	7.2–31.2

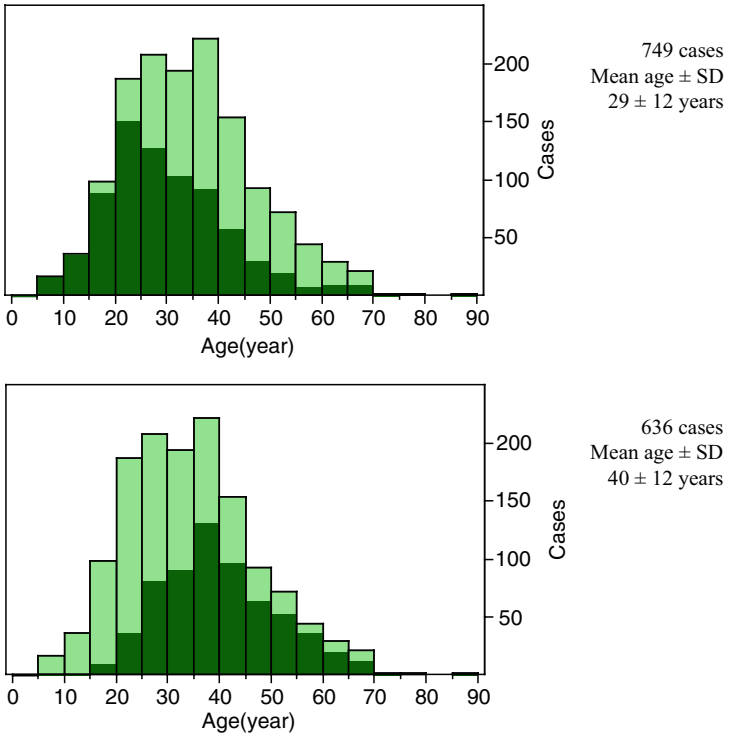

Fig. 27.1 Age at diagnosis and colorectal cancer in patients with FAP 1,300 cases in 900 families (Iwama T, et al. Int J Clin Oncol. 2004;9:308–316.): Top: Dark shadow means cases without cancer. Bottom: Dark shadow means cases with advanced cancer

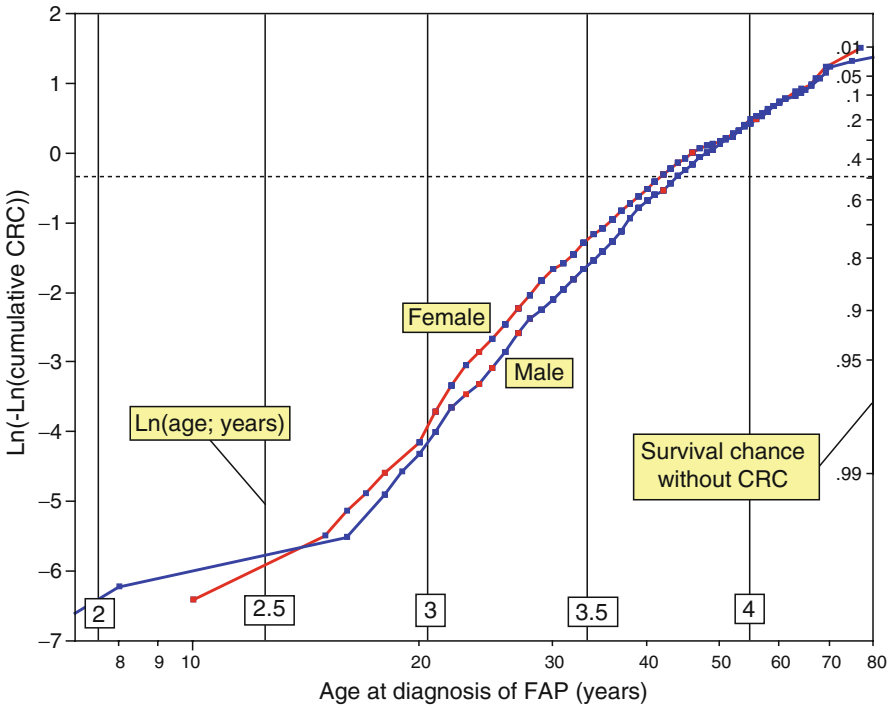

Fig. 27.2 Cumulative colorectal cancer in patients with familial adenomatous polyposis (Iwama T, et al. Int J Clin Oncol. 2004;9:308–316)

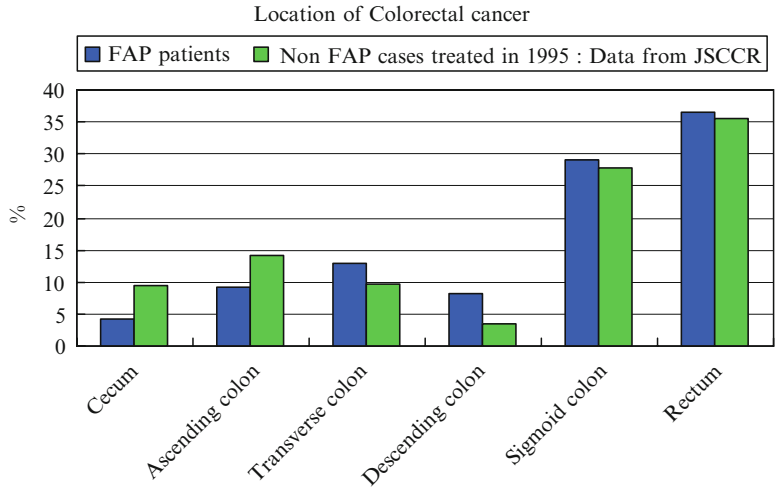

Fig. 27.3 Location of colorectal cancer (Iwama T, et al. Int J Clin Oncol. 2004;9:308–316)

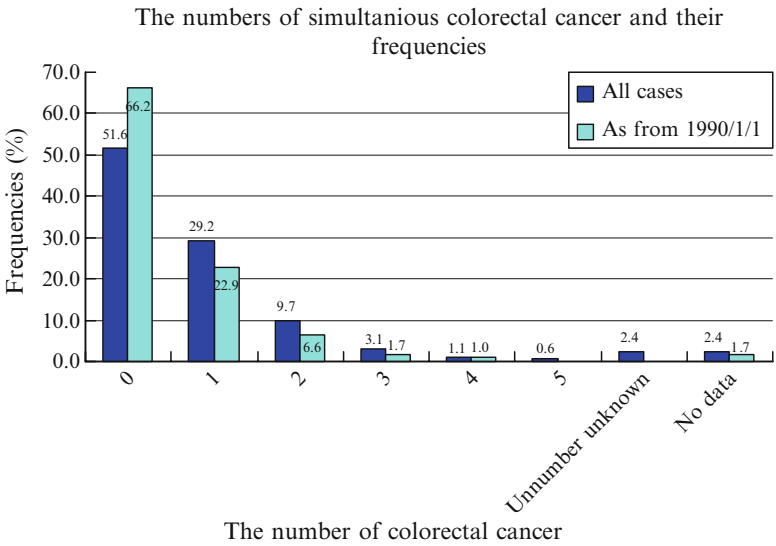

Fig. 27.4 Number of simultaneous colorectal cancer (Iwama T, et al. Int J Clin Oncol. 2004;9:308–316)

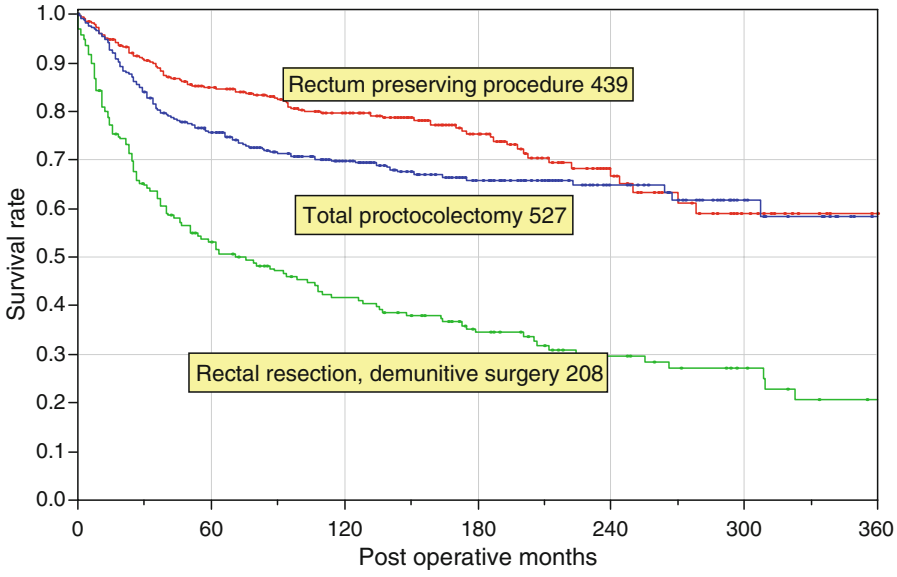

Fig. 27.5 Surgical procedures and their survival (Iwama T, et al. Int J Clin Oncol. 2004;9:308–316)

27.6 Patients' Association for Familial Adenomatous Polyposis and Their Family Members

Two patients' associations have been active in Japan for 11 years, one is in the East part (Tokyo) and the other is in the West part (Hyogo) of Japan. The purpose of these associations is (1) to have patients feel that they are not alone, (2) to facilitate communication with each other, (3) to acquire useful information for patients, (4) to improve their quality of life, (5) to encourage the research of hereditary colorectal cancer, and (6) make the nation know their situation. They have general meetings, recreation meetings, and lecture meetings. They issue newsletters several times a year. Sometimes they visit members of the parliament or welfare ministry office for lobbying. To date, only a small number of patients have joined these two societies because young persons cannot afford to join the activities or management of the associations. Several colorectal surgeons and genetic counseling nurses have joined the associations.

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Chapter 28
Across Culture and Health Systems: Africa

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Abstract Hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominant condition caused by mutations in the mismatch repair genes, accounts for 20–35% of inherited cancers and 1–7% of colorectal cancers (CRC). Colonoscopic surveillance reduces the incidence and mortality of CRC. Not all mutation-positive individuals comply with the recommended screening. The aim of this study was to identify factors that influence compliance. This qualitative study involved the division of mutation-positive individuals into a compliant and a non-compliant group. The study showed that a healthy, cancer-free life motivates individuals to comply with surveillance, while non-compliant individuals are unaware of or misunderstand reasons for regular surveillance. Barriers to compliance include the colonoscopy procedure, painful colonoscopy experiences of family members, ignorance about the increased risk of developing CRC and ignorance of the genetic test results. A clinical–genetic HNPCC service requires a cancer genetic counselor who highlights the importance of surveillance while addressing genetic properties of this preventable disease.

Keywords HNPCC • Colonoscopy • Surveillance • Compliance • Non-compliance

28.1 Introduction

HNPCC is an autosomal dominant inherited form of CRC which accounts for 20–35% of inherited cancers and 1–7% of all CRCs [1–6]. In South Africa, the estimated incidence for CRC in the indigenous African and Caucasian population

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group is three and 23 per 100,000, respectively [7]. The incidence in the Caucasian population resembles that of Westernized countries [8]. Disease-causing mutations in HNPCC are mostly found in the *hMLH1*, *hMSH2,* and *hMSH6* genes, which are involved in the cellular mismatch repair (MMR) mechanism [9–11]. Mutation rates in tumor cells with a deficient MMR system are 100–1,000 times higher than those in normal cells [12]. Prevention of deaths due to colon cancer in HNPCC families is possible through regular colonoscopic surveillance, which has the advantage of offering a 50–62% risk reduction and a 65% reduction in overall mortality [13].

Proposed guidelines for commencing surveillance are at age 20–25 years, or 10 years earlier than the youngest age of onset of cancer in the family, whichever is earlier, while colonoscopies should be repeated every 1–2 years until 30 years of age and annually thereafter [14, 15]. The relatively early age of onset (mean 42 years) and the predominance of right-sided lesions reported in HNPCC require more frequent surveillance than that required by the general population. Mutation carriers are advised to adhere to the surveillance while mutation-negative individuals are released from the regular HNPCC management and need only follow screening recommendations intended for the general population [16]. The recommended screening program is, by and large, conveyed by means of genetic counseling sessions and is a fundamental aspect of the HNPCC Predictive Testing Programme (PTP). Despite the demonstrated beneficial effects of CRC screening, compliance with recommended screening programs is less than optimal, notwithstanding the existence of a thorough PTP [13]. Published compliance rates vary between 50 and 80% in studies including first-degree relatives of patients with CRC, and from 63 to 93% in studies of HNPCC families [17]. A limited number of studies have investigated factors that play a major role in surveillance compliance and non-compliance concerning high-risk HNPCC individuals in particular (Table 28.1).

The Division of Human Genetics at the University of Cape Town (UCT) offers a PTP to families in whom a disease-causing mutation has been identified. In 2004, a total of 1,285 individuals in 351 families were recruited into the HNPCC research registry and the disease-causing mutation was identified in 29 of these families. Many of the mutation-positive individuals involved in the UCT PTP come from previously disadvantaged backgrounds and reside in rural under-resourced impoverished areas situated about 600 km (350 miles) from the nearest tertiary hospital. To overcome this

Table 28.1 Previously published factors affecting recommended screening guidelines compliance in high risk HNPCC individuals

- Perceived control over developing CRC [36]
- Physician recommendation for screening [5, 17]
- Being sedated [17]
- Embarrassment and discomfort of screening [17]
- Fear that a tumor would be detected during the screening [17]
- The absence of symptoms or other health problems [37]
- Low perceived risk of CRC [38]
- Misunderstanding predictive genetic test result leads to less worry about developing CRC [23]
- Younger age [5]
- Younger age associated with more discomfort [39]

problem, a specialist team including gastroenterologists, surgeons, pathologists, geneticists, and specialist colorectal registered nurses initiated an annual outreach mobile colonoscopic service approximately 15 years ago. The team travels to peripheral hospitals and clinics in these remote areas to offer the same quality of colonoscopic surveillance to individuals at high risk as they would have received at the tertiary hospital in the city [18]. Despite this being a comprehensive and free service, some individuals do not comply with their recommended surveillance.

28.2 Aim

The purpose of this qualitative study was to explore factors affecting surveillance compliance behavior in a group of mutation-positive individuals living in rural impoverished areas in the Northern Cape Province of South Africa.

28.3 Methods

The HNPCC Genetic database at UCT was searched for the colonoscopic surveillance profile of all mutation-positive individuals for the period 1991–2004, followed by non-random purposive sampling of participants to be included in the study.

The eligibility criteria require of the participants to:

1. Be mutation-positive with a genetic change in one of the MMR genes
2. Have participated in the UCT PTP during 1991–2004
3. Live in a geographically isolated part of the Northern Cape
4. Come from a socio-economically disadvantaged background
5. Be over 18 years of age at the time of entry into the PTP
6. Remember receiving their predictive genetic test results

28.3.1 Study Design

This was an exploratory, descriptive, cross-sectional, prospective study. A semi-structured interview schedule was developed. Closed-ended questions were used to obtain socio-demographic data and open-ended questions were used to encourage face responses allowing questionnaire items to be explored in greater depth [19, 20]. Content validity of the questionnaire was achieved by having an impartial person and two experts in HNPCC critically review the content of the questionnaire before the pilot study was conducted. This is a common procedure to establish content validity in qualitative research [21]. The measuring tool used was an interview schedule with a questionnaire of open- and closed-ended items.

28.3.2 Study Population

Participants were divided into a compliant and a non-compliant group by examination of clinic attendance records as determined from the time of receiving a predictive genetic test result. The compliant group included those who underwent surveillance at the recommended frequency. The non-compliant group included individuals who delayed having a colonoscopy at least once for more than 1 year, had a colonoscopy only once in their lifetime or never had a colonoscopy for a period of at least 5 years. A pilot study was performed using three participants (two compliant and one non-compliant) living within the Cape metropolitan area whose socio-economic background matched that of the research participants. As a result of the pilot study ambiguity, several questions were corrected and a number of questions were added to aid in-depth exploration of certain topics. Following an initial information session, the semi-structured interview focused on socio-demographic and economic concerns, the understanding of CRC status, family history of CRC, colonoscopy experience, worries about colonoscopy, and opinions regarding the cancer-genetic team. Interviews were conducted in either a private room in one of the local Primary Healthcare clinics or in the participants' homes and were recorded on audiotape. Follow-up group discussion was carried out once the themes from the interviews had been identified. During the group discussion, participants had the opportunity to introduce ideas not considered during the interviews. This qualitative study was approved by the Research Ethics Committee of the University of Cape Town (REC REF # 393/2004).

28.4 Results

During the period 1991–2004, the UCT Human Genetics Predictive Testing Programme has been offered to 15 of the 29 families in which a disease-causing mutation has been identified. A total of 221 of the 366 individuals who received their genetic test results were identified as mutation-negative. Overall, 60% (87/145) of high-risk mutation-positive individuals who received their genetic test results complied with surveillance recommendations. Application of the research eligibility criteria reduced the cohort of 145 mutation-positive individuals to 50 participants eligible for inclusion in this study. The remaining individuals were excluded as they were confused about their genetic test results. Furthermore, the majority of these non-eligible mutation-positive individuals (71/95) were non-compliant with the recommended surveillance.

Initially, verbal consent was obtained from 17 eligible individuals (six compliant and 11 non-compliant) and ultimately only eight (five compliant and three non-compliant) individuals provided written consent to participate in this research. Six of these participants received their predictive test results in 1997, while the remaining two participants received their results in 2003. The time from when blood was

taken during the initial counseling session to delivery of the genetic test result ranged from 1 to 3 years with the majority of participants (6/8) receiving their results within 1 year. Participants complied with the recommended surveillance over a period of 8 years (7/8) and 2 years (1/8). Surveillance behavior of non-compliant participants ranged from attending none, to attending three out of seven scheduled colonoscopies. Three participants had their first colonoscopy after their mutations status was ascertained while four participants were compliant, based on their empirical risk assessment, before they received their genetic test result.

28.4.1 Demographic Profile

Three males and five females of mixed ancestry in the age range 33–46 years participated in the study (Table 28.2). The highest level of education was Grade 12 (12.5%)

Table 28.2 Summary of participant profile (n = number of participants)

	Compliant (n = 5)	Non-adherent (n = 3)	Total (n = 8)
Sex			
• Male	2	1	3
• Female	3	2	5
Age[a]	39 years	38 years	39 (33–46) years
Marital status			
• Married	4	0	4
• Divorced	0	3	3
• Separated	1	0	1
Participants with children	5	3	8
Number of children[a]	2	2	2 (1–3)
Education			
• Grade 12	0	1	1
• Middle (Grade 7–11)	2	3	5
• Low (<Grade 7)	2	0	2
• Qualification after school	0	0	0
Employment status[b]			
• Full-time employed	2	0	2
• Housewife	1	0	1
• Unemployed	2	2	4
Household income[c]			
• R801–R1,600	0	1	1
• R1,601–R3,200	1	0	1
• R3,201–R6,400	1	0	1
• R6,401–R12,800	2	0	2

[a] Average value and range in brackets

[b] Not applicable to non-compliant participant who was in jail, n = 7

[c] Not applicable to non-compliant participant who was in jail, and two participants who did not know their household income/month, n = 5

while most (75%) participants completed Grade 7–11. Four unemployed female participants had family members who were employed and generated a monthly household income. The socio-economic status of the participants was low with the two most economically disadvantaged participants living in a household with an income of R2,000 (equivalent to approximately US$250) or less per month and supporting six people each. Although the remaining three participants had a higher income, they nonetheless supported three to four people, respectively. The cost of surveillance and transport to the Primary Healthcare hospitals where screening takes place are compensated for by a mining company or by the Provincial Health authority. Screening is offered in three towns in this geographical area and the reported distance (in time) from the participants' residence ranged from 5 to 90 min. Most participants (6/8) correctly stated that the transport was free of charge.

28.4.2 Themes Identified

Two major themes influenced compliance to surveillance – service and disease. *Service* refers to the genetic service, the surveillance service as well as the participant's understanding of these services, while *disease* represents issues regarding personal and family history of cancer, fear, and concerns related to cancer and social stigma associated with cancer. Specific factors impacting on compliance and non-compliance are summarized in Table 28.3. A selection of responses is listed below.

28.4.2.1 Service

Genetic Service

All participants found the CRC team to be helpful and friendly. However, one non-compliant participant considered the explanation of genetics offered as beyond her

Table 28.3 Summary of the themes that emerged from interviews and follow-up discussions

Service	Compliant group [$n=5$]	Non-compliant group [$n=3$]
1. Genetic service		
Recollection of counseling and result-giving dates	0	1
Remembered geneticist who counseled and delivered result	2	2
Knew genetic test result was positive	5	3
First attended colonoscopy:		
• Before counseling	0	1
• After counseling	4	0
• After result given	1	2

(continued)

Table 28.3 (continued)

Service	Compliant group [$n=5$]	Non-compliant group [$n=3$]
2. Surveillance service		
Reasons for compliance:		
• Knowledge health and cancer status	5	N/A
• Treatment or surgery options	3	N/A
• Experienced CRC deaths in family	2	N/A
Influenced by family members:		
• Compliance	1[a]	Did not mention
• Non-compliance	2[b]	Did not mention
• Painful colonoscopy experience	Did not mention	3[c] 2[d]
Personal experience of colonoscopy:		
Before colonoscopy:		
• Nervous or anxious	3[e]	2[e]
• Disliked preparation	4	3
• Preparation worst/one of worst parts of colonoscopy	2	2
• Preparation as *reason for non-compliance*	N/A	2
During colonoscopy:		
• Discomfort	5	3
• Discomfort was worst/one of worst parts of colonoscopy	3	2
• Discomfort as *reason for non-compliance*	N/A	2
• Painful	4	0
• Embarrassed	2[f]	0
• Sedative decreased pain and/or discomfort	4	1
After colonoscopy:		
• Relief	5	3
• Cramps	4	0
Suggestions to improve colonoscopy:		
• No preparation	Did not mention	2
• Stronger sedative	4	2
• No waiting time before colonoscopy	1	Did not mention
• Specific gastroenterologist to perform colonoscopy	2	Did not mention
• Allow participant to have colonoscopies at his convenience (*reason for non-compliance*)	N/A	1
3. Understanding of service		
• Reason for annual surveillance	5	0
• Purpose of surveillance	5	2
• Importance of surveillance vis-à-vis genetic test result	4	1[g]
Disease		
Awareness of CRC		
• Parent died of or developed CRC	2	1
• Parent died of cancer	1	0

(continued)

Table 28.3 (continued)

Service	Compliant group [$n=5$]	Non-compliant group [$n=3$]
• Family members died of CRC	0	1
• During first counselling session	2	1
Awareness being at risk of developing CRC		
• Informed by parent with CRC	0	1
• First counselling session	5	0
• Result-giving session	0	1
Fear of cancer – initial CRC discovery [n = 5]		
• Accept and handle it	3	2
Fear of cancer – recurrence of CRC [n = 3]		
• Not handle it	1	0
• Upset	1	0
• Did not realize CRC recurrence possible	0	1
Fear of developing CRC	2	0[h]
Stigmatization of cancer	2	2

[a]Motivated participant to comply
[b]Motivated one participant and discouraged one participant
[c]Discouraged all to comply
[d]Reason for non-compliance
[e]All females
[f]Only during first colonoscopy
[g]1 did not mention
[h]2 did not mention

level of understanding and this left her feeling confused and scared. Although participants referred to their result as "positive," they demonstrated different levels of understanding of the concept "positive." Most explained their positive genetic test result as being indicative of an increased risk of developing CRC. Their risk perception ranged between 70 and 99% to more than 50% or merely stating that they were at a high risk of developing CRC. Some compliant participants explained their positive result vis-à-vis surveillance, without referring to risks, or as a means of being proactive regarding their health, especially considering the experience of relatives dying of CRC. One participant was not surprised that she had the "mistake" since her father had died of cancer a month after she was born. She expected to be mutation-positive.

Surveillance Service

Compliant participants felt more comfortable and less scared before and/or during the colonoscopy procedure as a result of the interaction with the team. They understood why they should attend regular surveillance while the non-compliant participants apparently did not. Non-compliant participants were either confused regarding their risk of developing CRC or confused by the fact that a relative died of breast cancer

rather than CRC. They were thus unsure as to why they should be going for surveillance of the colon and not of their breasts. All except two non-compliant participants understood the principle of annual surveillance >30 years of age. In addition to being unsure as to the purpose of surveillance, two non-compliant participants were also confused regarding the actual colonoscopy procedure itself. One was unsure about the entry point of the colonoscope believing that it was through the mouth or the breast and that the colonoscope was checking for a fungus while the other described the colonoscopy being the pipe they put down his throat (referring to a gastroscope). Female participants explained their nervousness and anxiety before the colonoscopy in the context of having to wait for the procedure to be done. The longer they had to wait the more anxious they felt. Preparing for the colonoscopy was the worst, or one of the worst, part(s) of the whole process with complaints of nausea mostly due to the volume of fluid they had to consume. One participant vomited as a result of drinking the preparation. The discomfort during the colonoscopy was attributed to the wind that was pushed into the colon during the procedure leaving them feeling bloated and experiencing cramps. The pain experienced during colonoscopy was described as tenderness or cramps. One participant was not sure as to whether fear caused her to feel pain and/or tenderness during the procedure. The request to have a specific gastroenterologist performing the colonoscopy was based on feeling more comfortable with this person. This contributed to a relaxed feeling and thus feeling less pain. Changes recommended by participants toward a pleasant surveillance experience include a tablet form of preparation rather than the liquid, a stronger sedative and reduction in the waiting time before the procedure. Conversely, one compliant participant concluded that the colonoscopy could not be improved as it was an efficient functional procedure. He acknowledged that the procedure was painful but stated that this could not be changed. Three of the participants (two compliant and one non-compliant) underwent CRC surgery. Annual flexible sigmoidoscopy screening was recommended to all three of these participants postoperatively.

Understanding of Service

All five compliant participants were health conscious and went for colonoscopies on an annual basis in order to keep abreast of their cancer status. Knowledge of their cancer status gives a sense of control and going for colonoscopies is associated with staying alive to take care of children. The two compliant participants who had surgery remained in the recommended flexible sigmoidoscopy surveillance program. For them, the advantage of compliance versus non-compliance was to know whether they had cancer for which there would be an opportunity for treatment. All non-compliant participants had different reasons for not following the surveillance guidelines. Their reasons were based on the procedure itself, the preparation they had to drink before the procedure as well as the discomfort during the colonoscopy. Traveling distance as well as restrictions regarding access to a specific town where he would have liked to undergo his colonoscopy prevented a non-compliant participant from surveillance. Over and above their personal reasons for complying or not

complying, participants were to a certain degree influenced by the surveillance behavior of family members.

28.4.2.2 Disease

Awareness of CRC

This theme provided insight into awareness of CRC in families as well as the first realization of being at risk of developing the disease. Three participants (two compliant and one non-compliant) first became aware of a family history of liver and CRC at the ages of 22, 27, and 30 years respectively. The non-compliant participant, who had never had a colonoscopy, described the experience of shock when he realized that he had CRC while the two compliant participants anticipated the detection of cancer following their fourth and sixth colonoscopies, respectively. Worries about pain and recovery post-operatively rather than detection of cancer were more prominent for these two compliant participants.

Fear of Cancer

Fear of developing colon cancer did not occur for one compliant participant because she took solace in the fact that she "had Jesus in my life," while other compliant participants concluded that should cancer be detected the doctors could provide treatment. The compliant participant who already had one operation due to cancer knew what to expect and was less worried should he have to undergo surgery due to recurrence in the future. The only non-compliant participant who mentioned fear as a factor influencing surveillance behavior stated that she had no fear of developing CRC because many people had cancer. She would accept the diagnosis and not be afraid if she too developed it.

Stigmatization of Cancer

Stigma due to cancer influenced compliance of participants. One compliant and two non-compliant participants would only discuss their genetic test result with close members of their immediate family but would never share this information with other people in the community. They believe that the community would "gossip" about them and not keep it confidential. Another non-compliant participant explained that the community believed that cancer was somehow contagious stating that if someone had cancer people would not eat or sleep with them. An additional dimension related to stigma of cancer was awareness of cancer within the community. The community apparently distinguished between families with and families without cancer. Some participants *proudly* informed the researcher that their specific clan name (surname) distinguished those who had the familial colorectal cancer

from those who did not have the cancer. The medical staff in the clinics also used this clan distinction. One participant recalled that doctors only referred him to a tertiary hospital for specialized treatment when they realized he was a member of the clan, and according to him, this recognition probably saved his life.

28.5 Discussion

The main aim of PTP is reduction of unnecessary worry among those with a low risk of cancer (mutation-negative) and identification of those with a high risk (mutation-positive) to promote preventative measures and reduce cancer-specific distress [22, 23]. An important goal of genetic counseling is therefore to facilitate comprehensive consideration of medical, psychological, and social issues related to genetic testing and clinical surveillance. Cancer-genetic counseling furthermore aims to facilitate adjustment to proactive health behavior while promoting behavior consistent with medical recommendations. The genetic counselor should take into account that preventative options can substantially reduce the CRC incidence and mortality [5]. A counseling model based on a medical recommendation that also recognizes the patient's role in the decision-making process may be the most appropriate [24]. Promoting the individual's sense of control of the situation and confidence in the effectiveness and necessity of regular surveillance, leading to early detection of polyps, can reduce the perceived threat associated with HNPCC-related cancers [25].

The HNPCC team intuitively expected a different set of factors to influence surveillance behavior of compliant individuals compared to the set of factors influencing surveillance behavior of non-compliant individuals. However, this study illustrated that the same factor can be implicated in either compliant or non-compliant surveillance behavior. Factors, classified as cognitive–emotional, logistic and health systems related, include prolonged healthy, cancer-free lives, aspects of the colonoscopy procedure, surveillance experiences of family members, ignorance of increased risk of developing CRC, and being oblivious of genetic test results. Furthermore, these factors are not unique to HNPCC mutation-positive individuals but also exist and impact on compliance with recommended colonoscopy screening in the general ≥50 years at-risk CRC population, who are referred for their first screening colonoscopy [26].

The socio-demographic/socio-economic status of mutation-positive individuals cannot be ignored as predictors of surveillance-compliant behavior [26]. Compliance is more often than not compromised when individuals are from a resource-poor, disadvantaged background. In this study, the community is burdened with aspects of poverty, and this taken together with family and social concerns dominates participants' lives more than trepidation about their health. In many cases, problems of survival and family strife are central and more real than the threat of developing cancer. Since a preventative service like colonoscopic surveillance of mutation carriers is expensive, the clinical-genetic team is likewise challenged to sustain such a service

in a resource-poor country like South Africa [27]. Furthermore, surveillance services are restricted to main centers in South Africa further exacerbating the challenge of regular management of high-risk HNPCC family members from rural areas [18].

Over and above, the socio-demographic or socio-economic factors posing as predictors of compliance experience of cancer in the family may increase the psychological vulnerability of the genetically predisposed individual to a level of severe distress [28]. This could explain why none of the participants had colonoscopies prior to their contact with the clinical-genetic team despite their awareness of familial cancers. This distress is even apparent in mutation-negative relatives. They insist on having annual colonoscopies despite persistent genetic counseling, during the outreach mobile colonoscopy service, reiterating their negative mutation status. High levels of distress before genetic testing is a key predictor of post-test anxiety, as found in testing for the BRCA gene in breast cancer [29]. Adverse anxiety subsequent to testing is evident [30] and can possibly be addressed through reduction of pre-test distress [25] with a subsequent impact on surveillance behavior. Despite the years of contact with the clinical/genetic team, a mere 75% of the participants, an equal number of compliant and non-compliant individuals, understood the meaning of their genetic test result, albeit at differing levels. Differences might exist between an individual's perceived risk interpretation vis-à-vis his/her genetic test result and the eventual acceptance of such a risk [26, 31]. Furthermore, Bjorvatn found that although perceived risk declined following genetic counseling, incongruence continued regarding expression of risk in words and in percentages [32]. None of the non-compliant participants in our study understood the reason for attending annual surveillance while all the compliant participants did. A mere 73% of patients in Norway correctly considered their inclusion in a surveillance program following a genetic counseling session [32].

Besides the reported influence of painful colonoscopy experience of mutation-positive individuals on compliance [17, 33] this study also identified the influence of painful colonoscopy experiences of family members on compliance. The logistics of how the outreach service operates could explain why experiences of family members impact on compliance. Groups of individuals are transported from their respective residences to one of the three clinics or hospitals where the surveillance service is offered. Participants reported that traveling time consequently presented the opportunity to revisit and discuss colonoscopy experiences of the previous year leaving them not only distressed as to what is waiting for them, but also distressed about what their family members are going through and, more importantly, refuse to endure. Thus, over and above their psychological vulnerability regarding familial cancer, they also experience distress related to the recommended surveillance program.

It was not anticipated that social stigma of cancer would present as a possible factor impacting on surveillance behavior in this study. Stigmatization suggests that the individual has an unwanted abnormality (in this case, cancer) and is therefore disqualified from being fully socially acceptable [34]. The consequence of the stigma is emphasized during social interactions between those who are stigmatized and those who are not. These interactions are generally negative (anxiety, disgust, sadness, anger, or helplessness), but may also have positive aspects like empathy or

over-concern. Both these types of responses suggest the attitude that the stigmatized person is unfavorably different from "normal" people [34]. In this present study, two dimensions concerning stigma were identified. The first dimension was a negative trait of the community and was a result of the ignorance of people vis-à-vis cancer. Participants indicated that the community believed that cancer was contagious. This is similar to the situation in West Bengal where respondents (21%) expressed the idea that cancer was an infectious disease creating a problem of isolation from the family or society for cancer patients [35]. The second dimension was a distinction between families with cancer, and families without cancer, based on family name. In this study, social stigma of cancer had a significant bearing on the PTP and consequently on surveillance behavior of mutation-positive individuals. Family system characteristics contribute to cancer distress and this could have an impact on self-regulatory health behavior [28].

Research participants recommended the following to improve the clinical-genetic service delivered in this resource-poor area: privacy after the colonoscopy procedure, a stronger sedation, a 6-month follow-up with a genetic counselor after the post-test result-giving session, constant follow-up of individuals who do not manage with or show misunderstanding regarding surveillance or genetic information. They also suggested that a support group be initiated within the community to give people the opportunity to talk about their concerns relating to CRC.

Providing families with the opportunity of genetic testing and surveillance comes with a responsibility for the genetic team and the families to whom these services are being offered. The genetics team has the responsibility to educate individuals at their level of understanding thus empowering them to take ownership for their new-found knowledge in such a way as to sustain their health. Furthermore, factors affecting compliance with surveillance go far beyond the physical screening procedure. Mixtures of psychological, attitudinal, cultural, and socio-economic aspects influence compliance behavior. A strategy to maximize surveillance will thus need to take the individual within the context of their family, community, and culture into consideration. "Although 100% compliance with screening recommendations is probably not a realistic goal, any incremental increase in compliance rates will hopefully translate into reduced morbidity and mortality in this vulnerable population" [17].

28.6 Conclusion

The aim of this study was to explore factors affecting surveillance compliance behavior in a group of mutation-positive individuals. Various yet similar factors impact on both compliance and non-compliance with recommended surveillance guidelines. The development of our understanding of molecular genetics creates possibilities to diagnose and also to determine susceptibility to inherited cancers. Delivering a PTP service in the rural, impoverished areas of the Northern Cape allows families to benefit from such understanding and have access to a service that would otherwise be unaffordable and inaccessible to them. The logistics of

delivering such a service adds complexity to the inevitable challenge of clinical-genetic intervention in South Africa. Furthermore, the transition from genetic research to clinical service has not only opened up unique service opportunities, but also unique psychosocial situations for affected families. In a developing country such as South Africa, tribulations related to poverty, low education level, and unemployment have some bearing on genetic service delivery. Factors contributing to compliance with recommended surveillance are therefore not only associated with the behavior of the mutation-positive individual.

Further research should be expanded to all non-compliant individuals in South Africa. More importantly, unearthing the fact that 95 mutation-positive individuals were excluded from the study due to confusion about their genetic test results begs the question how this contributes to compliance to recommended surveillance. Over and above, considering how confusion impacts on surveillance behavior research as to how confusion relates to the genetic counseling service delivery should be considered. Furthermore, investigation is needed regarding different coping styles and health belief models of mutation-positive individuals. The inevitable role of the genetic counselor specifically in the multidisciplinary team is at the center of all these investigations.

Limitations of the study

- The sample size of eight individuals and the ethnic homogeneity might have led to incorrect inferences and may limit the degree to which the study results can be generalized
- Researcher Bias: The participants' responses might have been what they thought was appropriate rather than their true attitudes
- Rumination Bias: The participants were confronted with questions to which they previously had not given much consideration
- Selection bias: non-compliers were over-represented among those who declined to participate in the study, which is a common limitation in compliance studies

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Chapter 29
Across Culture and Health Systems: Argentina

Carlos A. Vaccaro

Abstract Argentina is a developing country with unevenly distributed health resources and no professionals trained to perform genetic counseling for hereditary cancer. According to the available data, cancer represents a 20.7% of all causes of death and it is the second leading cause of death after cardiovascular diseases in both sexes. Colorectal cancer (CRC) is the third commonest tumor only overtaken by breast and cervical cancer uteri cancers in women, and lung and prostate cancers in men. According to the International Agency for Research on Cancer there are 10,900 new cases of colorectal cancer diagnosed each year leading to incidence rates per 100,000 of 30.1 for males and 19.1 for females.

As most of the countries in South America, Argentina does not have a national cancer registry. Experience in counseling for hereditary cancer is limited to the only 3 Argentine registries which are concentrated in urban centers and have a restricted scope, determined largely by the interest of individual clinicians and researchers and by resource limitations. Data from our Registry (ProCanHe) showed 79% of the individuals were aware of their risk when a close relative died from (34.5%) or were diagnosed with (44.5%) CRC. Only 1.2% was warned by a physician. Eighty-one percent of them had received surveillance recommendations. However, this occurred at a mean age of 29 years. Before counseling, up to 73% of participants had heard little or nothing about genetic testing for cancers. In this scenario, international collaboration allowed the implementation of genetic testing with a high degree of satisfaction. In 2004, in Buenos Aires, a Regional Meeting of the Collaborative Group of the Americas on Inherited Colorectal Cancer was held with the attendance of the South American leaders. Two years later, in Sao Paulo, a South American group called "Grupo de Estudio de Tumores Hereditarios (GET) was founded on the initiative of Benedito Rossi. Currently, the first collaborative study is ongoing.

However, support for continued surveillance and counseling is still limited, which makes it difficult to appropriately run the registries.

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Keywords Lynch Syndrome • Colorectal cancer • Developing country • Health system • Cancer mortality • Genetic counseling • Hereditary cancer registry

29.1 Overview of the Economic, Social, and Health Situation

Argentina is the eighth largest country in the world and the second largest in South America after Brazil. For administrative purposes, it is divided into 23 provinces and the city of Buenos Aires, which is considered a federal district. The physical characteristics vary widely between the provinces. According to the last census, the population is 38,226,051 inhabitants, with 88% of them living in urban areas and 30% in greater Buenos Aires [1]. In contrast with a high rate of literacy (97.4%), a high proportion of the population lives in poverty or indigence (31.4 and 11.2%, respectively). However, after the economic crisis in 2001, the economy has been growing 8% annually for 4 consecutive years, with less than 10% of inflation rate and less than 10% unemployment.

The human development index is 0.788 and the life expectancy at birth is 71.93 (75.5 and 68.4% for women and men, respectively), which is almost 2 years less than the worst estimation in Europe [2]. Child mortality is twice as much as the worst seen in Europe. In relation to Chile and Uruguay, all these figures are worse.

The proportion of people over 60 years old (13.31%) is increasing but is still lower than that in developed countries (which ranges from 18 to 24%). However, compared with other South American countries, this proportion is higher (i.e. Chile: 10.37%, Brasil: 7.95%) [3].

By 2004, the fiscal expenditure on health was estimated at 7.3% of the GDP. Although this nears the median seen in Europe (8.5%), it should be taken into account that the average GDP in Latin America is approximately 10% of that of industrialized countries [4, 5]. In this way, although the annual per capita health expenditure comes to US$ 250 (which is slightly higher than the US$ 105 average in Latin America), it is 7.4 times less compared with that of US$1,860 of industrialized countries [6].

The health system has two main components: public and private. The public health system provides medical care through 1,271 hospitals and 6,456 primary care centers, most of them lacking high technology [7]. As a consequence of the economic crisis (which led to an increase in unemployment and illegal work), between 1997 and 2001, the proportion of the population having access only to the public health system increased by an 18% [8], reaching as much as 48.1% of the population (26.2–65.5% in the different provinces). A recent survey showed that the main complaints of people in the public system include: lack of attention (18%), bad performance (13%), limited coverage (12%), bureaucracy (7%), and additional payments (6%). Social security represents 46% of the health system and is associated with a satisfaction degree ranging between 46 and 82% (among nonusers and users, respectively). However, many

workers pay for additional medical care, thus reaching a 92% rate of satisfaction. Finally, 10% of the population has access to private insurance (most of them being able to receive medical attention similar to that in developed countries), which, in terms of the total of the health expenditure, represents up to 46% of it.

Health resources are unevenly distributed: the ultra specialization and high technology available in some medical centers contrasts sharply with the lack of basic resources for medical attention and prevention at many primary care centers. The privatization of health care that occurred in the last decade is increasing the inequities [9, 10].

Regarding health care professionals, there are 26 Schools of Medicine (16 private, 10 public). This extremely wide offer makes access almost non-restricted, yielding more than 4,000 new professionals annually. With one physician for every 310 people (four for every one nurse), Argentina holds one of the highest ratios of physicians to patients in the world. However, the distribution of both medical centers and doctors is unequal, with more than 50% of them in Buenos Aires. Therefore, a great proportion of physicians are not able to practice.

Argentina does not train health professionals to perform genetic counseling, which remains a practice restricted to geneticists (mainly limited to birth defects). Experience in counseling for hereditary cancer is limited to the only three Argentine registries: ProCanHe, at the Hospital Italiano de Buenos Aires (private center), the Registro Hospital Bonorino Udaondo (public center) and Registro de Prevención en Cáncer Colorrectal (RePreCC) at the Hospital Santojanni (public center).

29.2 Genetic Services in the South America: Collaboration Is Beginning

A shared limitation of South American countries is the lack of human resources trained in clinical genetics, genetic counseling, and laboratory genetics. [11] Although genetic testing for people at risk has been proven to be cost effective even in developing countries [12], registries do not receive financial support either from the health system or from private organizations. As a consequence, these programs lack permanent financing and skilled personnel, limiting medical services and the capability to conduct scientific research. Currently, the testing to search mismatch repair system defects is limited to microsatellite instability and immunohistochemical analysis (approximate cost in Argentina: US$ 400 and US$ 100, respectively). The search for a mutation related to any hereditary cancer is limited to research laboratories with no commercial laboratory providing genetic testing. The high cost of processing the samples in North America or Europe is not affordable for most of the people and health systems. Therefore, the only cases in which a mutation was searched were those included in research collaborative studies.

Nevertheless, the increasing awareness of medical professionals and of the public at large about the role of genetic factors in cancer make it mandatory to improve the resources of the registries and to establish networks of collaborating laboratories so as to avoid duplication of services, to reduce costs, and to perform quality controls. Although the World Health Organization has supported the development of genetic services in developing countries [11, 13–15], genetic services for hereditary colorectal cancer have only had an incipient and fragmentary development in Latin America. Specifically, there are only seven registries in South America: three in Argentina, two in Brazil, one in Chile and one in Uruguay. The Grupo Uruguayo de Tumores Hereditarios, led by Carlos A. Sarroca**,** is the oldest one.

These services are concentrated in urban centers and have a restricted scope, determined largely by the interest of individual clinicians and researchers. Reasons for the slow development of genetic services in the region include: a burden of unmet needs in other areas of health (e.g., infectious diseases, malnutrition, etc.) and misconceptions about cost-benefit of genetic testing. [12]

In line with the lack of governmental support for biomedical and epidemiological research in general, and the scarcity of resources and funds, genetics research in the region is far from the potential level it could have according to the abilities of South American scientists. Fortunately, international collaboration is beginning. In 2004, in Buenos Aires, a Regional Meeting of the Collaborative Group of the Americas on Inherited Colorectal Cancer was held with the attendance of the South American leaders (Francisco Lopez-Kostner from Chile, Carlos Sarroca from Uruguay and Raul Cutait and Benedito Mauro Rossi from Sao Paulo Brazil). Two years later, in Sao Paulo, a South American group called “Grupo de Estudio de Tumores Hereditarios (GET) was founded on the initiative of Benedito Rossi. Currently, the first collaborative study is ongoing.

29.3 Cancer Figures

As with most of the countries in South America, Argentina does not have a national cancer registry. Alternatively, nine regional registries work in seven countries. Two of them are located in Argentina: the oldest one in Concordia, province of Entre Ríos and another one in Bahía Blanca, province of Buenos Aires. Last year, with the support of the World Bank, ten additional regional registries began working, but without national integration.

Besides data from regional registers, the national official figures are estimated on information from death certificates, making them inaccurate. According to the available data, cancer represents a 20.7% of all causes of death and it is the second leading cause of death after cardiovascular diseases in both sexes with an age-adjusted mortality rate per 100,000 of 144.19. During the last 10 years, this rate has shown a decrease tendency of −1.28% and −0.22 in women and men, respectively. It has been estimated that around 10% of cancer mortality in Argentina is related to the medical health system.

Colorectal cancer (CRC) is the third commonest tumor only overtaken by breast and cervical uteri cancers in women, and lung and prostate cancers in men. According to the International Agency for Research on Cancer, there are 10,900 new cases of colorectal cancer diagnosed each year leading to incidence rates per 100,000 of 30.1 for males and 19.1 for females. [16] These are lower than North American rates (44.6 and 33.1, respectively). Compared with other South American countries, only Uruguay**,** with rates of 39.6 and 29.5, respectively, has a higher incidence. Most of the other countries of the region have half of this incidence (i.e., Chile: 15.8 and 15.1, respectively; Brazil: 14.4 and 14.3, respectively, Colombia: 11.7 and 14.6, Venezuela: 11.2 and 11.6, respectively, Ecuador: 7.6 and 10.0 respectively, Perú: 11.7 and 12.3, Paraguay: 10.3 and 9.0, Bolivia: 15.9 and 8.5, respectively).

With 6,285 deaths per year (colon cancer: 5,460, rectal cancer: 825) affecting both sexes equally, CRC also represents a common cause of death (the third in males after lung and prostate, and the second in females after breast cancer) with age-standardized rates of 14.7 for males and 9.8 for females.

Although in 2005 a national consortium integrated by the most important medical and surgical associations adhered to the American strategy for colorectal screening, no official cancer prevention or control program has been implemented yet. Furthermore, no specific strategy has been considered for hereditary cancers yet.

29.4 Register History and Characteristics

In 1970, headed by Fernando A. Bonadeo M.D., a colorectal section was organized in the Service of General Surgery. Currently, this section is integrated by five surgeons and keeps a prospective database of over 4,000 colorectal cases, which represents the largest one in Argentina and one of the largest in Latin America. In 1996, an institutional registry on hereditary colorectal cancer (Programa de Cancer Hereditario, Pro.Can.He.) was founded by C. Vaccaro becoming the first one in Argentina focused on HNPCC. One year later, its experience with nine families was published as the first report from a registry in Argentina. [17] Currently, the registry has data from 57 families fulfilling Amsterdam Criteria, 20 fulfilling modified Amsterdam Criteria, 391 familial cancers, 26 families with familial adenomatous polyposis, ten patients with Peutz–Jeghers and two with juvenile polyposis.

Regardless of limitations already stated, the Registry has grown steadily mainly owing to personal efforts and the collaboration of several foreign centers. A crucial moment for the development of the registry was the meeting with Henry T. Lynch during his visit to Uruguay in 1998 to counsel an Uruguayan family (Fig. 29.1). By then, a collaborative study was initiated with his help and that of Paivi Peltomaki MD.

Fig. 29.1 Henry T. Lynch, Jane Lynch and Carlos Sarroca with the members of the Uruguayan family at the Hospital Militar, Montevideo, Uruguay

Since no center had experience with genetic counseling on HNPCC in Argentina, the visits to Registries at Cleveland Clinic Foundation (with James Church and Ellen McGannon) and MD Anderson (with Patrick Lynch) allowed our registry to gain knowledge and carry out counseling in an appropriate manner. As a consequence of this collaboration, as well as the continued help and support received from Terry Berk, several Argentine families could undergo genetic testing and have been counseled. Additionally, several research studies could be accomplished and published [18–20].

29.5 The First 10 Years of Experience

Our experience with epidemiological, molecular and counseling data was recently published in the *Disease of Colon and Rectum [*20]. Here we present an update over 57 families registered from 1996 to 2007 fulfilling the Amsterdam criteria. These cases were identified from our historical database (upon physician suspicion or early onset of cancer) and more recently among people referred or self-referred to the ProCanHe. Pedigrees were constructed upon family background obtained by personal interview with the proband. Confirmation by pathologic tissues or pathology reports was made whenever possible.

Those families which were eligible for genetic testing were counseled according to the Creighton University's recommendations in three stages. The pre-test stage was performed to provide information about all aspects of natural

Fig. 29.2 Carlos Vaccaro and Daniela Habsuda (psychiatrist) giving genetic counseling in a familial session at the Hospital Italiano de Buenos Aires, Argentina

history and current surveillance strategies as well as to discuss advantages, potential disadvantages, and limitations of genetic testing. According to the family preference, this first stage was done in an individual or familial information session (Fig. 29.2). For one family, the counseling session was done as a field visit at one member's home (Fig. 29.3). For the second stage (pre-result stage), the family members were contacted to personally and confidentially receive the results in an individual session during which all potential implications of genetic testing were discussed again with the assistance of a psychiatrist (Fig. 29.4). The last stage (follow-up) was performed to update information about the family background and to determine the degree of accomplishment of surveillance recommendations. At all the stages, participants were asked to complete a survey form including data related to knowledge about the disease and its psychological impact.

29.6 Results

A total of 57 families fulfilled the Amsterdam Criteria (45 AC I and 12 AC II). Eighteen (31%) presented as Lynch syndrome I, 36 (63%) as Lynch syndrome II, and 3 (6%) as Muir–Torre syndrome. Table 29.1 depicts their clinical characteristics.

Data from 839 relatives (53% females) of a mean of 46.4 (range: 2–94) could be obtained. The median number of individuals per family was 14 (range: 1–41) with no statistical difference between AC I families and AC II families (12 [IC95%:

Fig. 29.3 Carlos Vaccaro and Xavier Garione (colorectal fellow) during a field visit to Rosario (province of Santa Fe) in 2000

Fig. 29.4 Carlos Vaccaro and Daniela Habsuda during an individual session giving genetic counseling based on test results

9–24] vs. 8 [IC95% 4–28], respectively, $P = 0.10$). Families characterized as Lynch syndrome I presented a trend to have less relatives compared with families characterized as Lynch syndrome II (8 [IC95: 5–22] and 17 [IC95%: 12–25], respectively, $P = 0.09$).

Table 29.1 Characteristics of families fulfilling Amsterdam criteria (*n*: 57)

Phenotype	Number of relatives	Generations studied	Amsterdam CRITERIA	Number of CRC	Number of non CRC	Individual with more than 1 tumor
Lynch I	8	3	I	2	2	2
Lynch II	17	3	I	3	4	0
Lynch II	24	4	I	4	5	1
Lynch I	14	4	I	3	0	0
Lynch I	4	5	I	4	0	0
Muir-Torre	28	4	I	10	8	3
Lynch II	19	3	I	5	1	0
Muir-Torre	31	4	I	8	7	6
Lynch II	22	3	I	4	1	0
Lynch II	9	3	I	3	6	1
Lynch II	23	5	I	5	3	0
Muir-Torre	22	5	I	5	2	1
Lynch I	10	4	I	4	0	0
Lynch I	32	5	I	4	0	1
Lynch II	17	5	I	3	1	1
Lynch I	5	4	I	3	1	0
Lynch II	41	6	I	21	1	1
Lynch II	6	4	I	3	3	3
Lynch I	1	3	I	0	0	0
Lynch I	22	4	I	6	0	1
Lynch I	1	4	I	1	0	0
Lynch I	12	3	I	3	0	0
Lynch II	35	5	I	13	2	1
Lynch I	6	4	I	3	4	0
Lynch II	20	4	I	4	3	0
Lynch II	7	4	I	3	3	0
Lynch II	28	4	I	8	1	0
Lynch II	12	3	I	3	3	1
Lynch II	24	4	I	3	6	2
Lynch I	3	3	I	3	0	0
Lynch I	10	4	I	4	1	2
Lynch II	33	5	I	8	8	5
Lynch II	11	4	I	3	8	1
Lynch II	7	5	I	4	1	1
Lynch I	1	6	I	1	0	0
Lynch I	1	6	I	0	0	0
Lynch I	26	4	I	3	2	0
Lynch II	23	5	I	3	4	2
Lynch I	7	4	I	4	0	0
Lynch II	40	5	I	3	10	0
Lynch I	7	4	I	3	0	0
Lynch II	18	4	I	6	1	1
Lynch II	20	5	I	5	4	1
Lynch II	1	5	I	1	1	1

(continued)

Table 29.1 (continued)

Phenotype	Number of relatives	Generations studied	Amsterdam CRITERIA	Number of CRC	Number of non CRC	Individual with more than 1 tumor
Lynch II	1	4	I	1	0	0
Lynch II	9	5	II	2	7	0
Lynch II	28	5	II	1	9	1
Lynch II	8	5	II	4	5	1
Lynch II	4	4	II	2	2	0
Lynch II	7	2	II	2	1	0
Lynch II	1	4	II	1	0	0
Lynch II	7	4	II	3	6	3
Lynch II	10	4	II	3	2	0
Lynch II	26	3	II	4	5	0
Lynch II	12	3	II	3	1	2
Lynch II	13	4	II	6	3	0
Lynch II	5	4	II	1	2	0

CRC colorectal cancer; *Non CRC* non colorectal cancer

A total of 343 patients (52% females) with cancer were identified among the 839 relatives (40.8%). The median of affected members per family was six (IC95% 5–8, range: 3–24). In 210 cases (61.2%), CRC was the first diagnosed tumor and 42 (12.2%) developed more than one tumor (33 patients presented two cancers, five patients three cancers, and four patients four cancers). A paradigmatic case was a woman who developed an adenocarcinoma in the cecum at age 39. By this time, her family background, although included many affected members, did not fulfill the Amsterdam criteria and genetic testing was not available. She did not accept to undergo a prophylactic hysterectomy. One year later, she developed an adenocarcinoma of the endometrium. At age 44, she was operated on for a mucinous adenocarcinoma duodenal and at age 49 for a breast adenocarcinoma. This patient was found to carry a novel mutation (hMSH2: exon 12, del C en nt 1910, codon 637) and her family background showed a strong aggregation of breast cancer (Figs. 29.5 and 29.6). A lack of inmunohistochemical hMSH2 expression was found in all the tumors.

A total of 213 cases of colorectal cancer were identified with a median of 3 (IC95% 3–5, range: 1–21) cases per family. The mean age at diagnosis was 52.1 (range: 21–90) with 53% of the cases diagnosed before 50 years (highest prevalence between 41 and 50 years: 34.8%). This age of onset is similar to that reported from Europe [21] and slightly higher than those reported in Brazil [22–24]. in Uruguay [25, 26] and in North America [27, 28].

Age of onset was the same in cases identified by AC I and AC II (50 years) and was related to the generation number (Table 29.2). This anticipation of developing cancer could be explained by a secular time trend in cancer occurrence and or improvement in screening and surveillance strategies.

Patients with more than one tumor developed colorectal cancer at an earlier age: 45 (range: 21–87) vs. 51 (range: 22–90), $P = 0.001$.

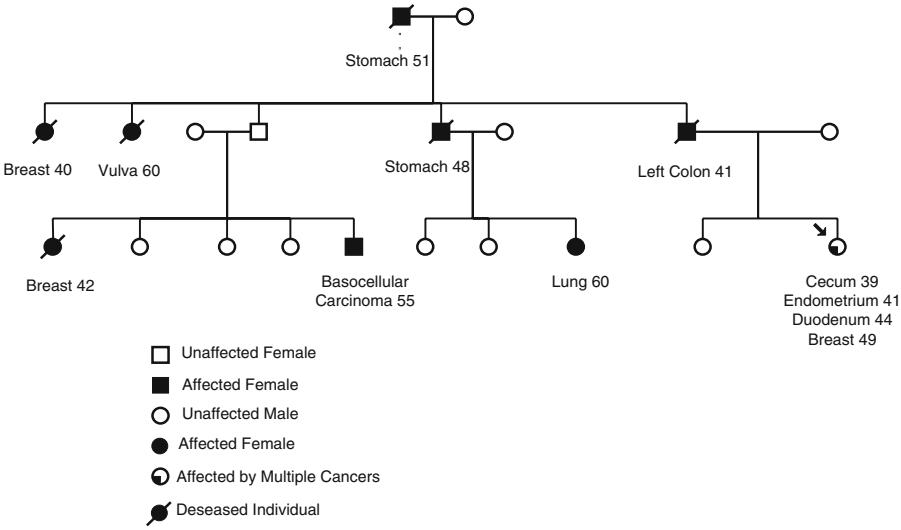

Fig. 29.5 Pedigree of the family with breast cancer aggregation in which a novel mutation in the MSH2 gene was found

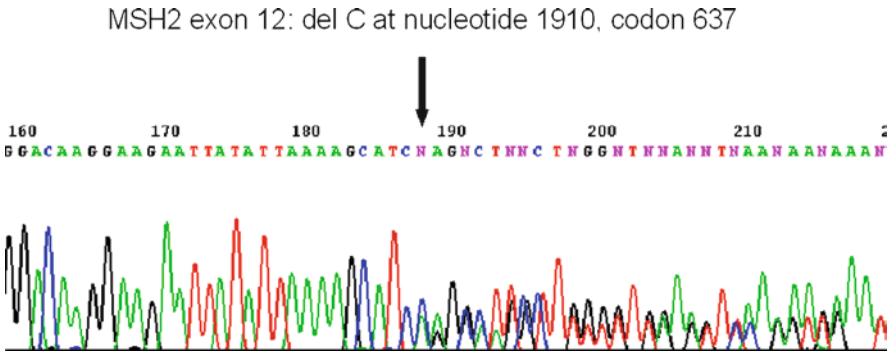

Fig. 29.6 The novel mutation in a family with breast cancer aggregation. Deletion of Cistina in exon 12, nucleotide 1910, codon 637 of the hMSH2 gene

Table 29.2 Age of onset for colorectal cancer cases in the different generations

	# Cases	Median age of onset	Range
Generation I	18	65	45–81
Generation II	87	55	23–90
Generation III	82	48	21–79
Generation IV	29	45.5	23–58
Generation V	9	42	22–45
Total	187	51	21–90

$P < 0.0001$

One-hundred ninety-six out of the 343 (57.1%) affected members presented colorectal cancer only, 26 (7.6%) associated with extracolonic tumors and 121 (35.3%) presented with extracolonic cancer alone. These relative proportions are similar to those reported in USA.

Regarding extra colonic tumors, breast cancer and gastric cancer were the most common tumors in females and males, respectively.

29.6.1 Genetic Counseling

Only 25 (43%) out of the 57 families fulfilling Amsterdam criteria could be counseled by the registry. This was mainly due to the inability of the registry to offer adequate support because of resource limitations. In the data regarding the 84 counseled relatives (57% females; mean age 44.7 years old [range: 18–81]), several figures point out the lack of knowledge of people at risk and the need of an educational register program:

1. Seventy-nine percent of the individuals were aware of their risk when a close relative died from (34.5%) or was diagnosed with (44.5%) CRC. Only 1.2% was warned by a physician.
2. Although 71% of the interviewed referred that their personal medical doctors knew about their family background, only 62% had shared information with them.
3. Eighty-one percent of them had received surveillance recommendations. However, this occurred at a mean age of 29 years. Furthermore, this information was provided by a physician in only 32% of the cases.
4. Before counseling, up to 73% of participants had heard little or nothing about genetic testing for cancers. This rate is higher than the 64% found within the American population by a NIH study [29].
5. Sixty-seven percent referred having new relatives showing interest in being counseled.
6. Ninety-seven percent of the relatives estimated that they would accomplish the surveillance recommendations provided by the registry.
7. After becoming aware of the oncological risk, 61% of the individuals expressed concern and 11%, fear. These figures are similar to those reported from Europe [30].

Regardless of the lack of experience in giving genetic counseling and the fact that the session was led by a surgeon (CAV), all counseled members considered the session adequately implemented, very useful, and recommendable for their relatives at risk. Sixty-six percent referred to feel better than before the session and 93% stated to trust in the confidentiality of the data. These results are as satisfactory as those reported by well-organized registers [31–33].

All the people eligible for molecular testing accepted to pursue testing. This is in accordance with a cohort study conducted at the NIH where 97% of the individuals

stated this intention [29]. In our series this high level of acceptance could be explained not only for the adequate implementation of the counseling but also by the fact that no fee was charged. An additional cause may include the lack of fear to discrimination by health insurance companies or by employers, which is the most frequent reason (up to 39%) to refuse testing in United States [29]. Among individuals who had no children, 46% identified their own health concern as the most important reason to consider testing. On the other hand, those with children identified learning about their children's risks as the most important motivation. Again, the figures above are similar to those reported among Americans.

Data from the United Kingdom show that most people still want screening if at low risk and would make more plans for the future if they were at high risk [34]. In our series, 52% of the individuals referred that the genetic testing results would modify their life style or future plans.

Although only 50% considered informing their physicians, as much as 87% of the people referred no concern or difficulty sharing the information with their relatives or friends (which is similar to other series) [35]. Furthermore, all individuals expressed feeling emotionally supported by their families or friends.

Among the people contacted for follow-up, 86% were willing to receive an additional counseling session and 38% would like to receive psychological support. Seventy-six percent of the people interviewed had talked about their cancer risk in the last year, mostly with their family members (71%) and/or with their family doctor (29%). Lack of interest and fear were the most common causes referred by those who had not recently discussed their risk. Ten out of 12 individuals who had children over 18 years had shared the information with them.

Regarding implementation of surveillance recommendations, 76 underwent a videocolonoscopy last year [36, 37]. Among women, 36 underwent surveillance (transvaginal unltrasound and ecography and serum CA125) last year. As worldwide reported, lack of interest and fear were the main reasons not to pursue surveillance.

29.7 Final Considerations

Argentina is a developing country with unevenly distributed health resources and no professionals trained to perform genetic counseling for hereditary cancer. Experience in counseling for hereditary cancer is limited to the only three Argentine registries which are concentrated in urban centers and have a restricted scope, determined largely by the interest of individual clinicians and researchers and by resource limitations.

This experience is unique in Argentina with few additional data from other Latin American countries. International collaboration allowed the implementation of genetic testing with a high degree of satisfaction. However, support for continued surveillance and counseling is still limited, which makes it difficult to appropriately run the registry. Although genetic testing for people at risk has been proven to be

cost-effective even in developing countries, registries do not receive financial support making these programs lack permanent financing and skilled personnel. Regional collaboration is beginning and promising.

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Chapter 30
Genetic Counselling Across Culture and Health Systems: Australia

Finlay Macrae and Clara Gaff

Abstract Australia is a multicultural society where the Anglo-celtic heritage and institutions dominate the culture of the country. In this chapter, the health care system, the centralization of cancer care and cancer registries, and cancer family registries will be discussed. A detailed discussion of the approach to genetic counselling including the training of genetic counsellors, research, and privacy issues among others will be included.

Keywords Genetic counselling • Hereditary colorectal cancer • Surveillance • Genetic testing • Cancer registries • Family registries

30.1 Multi-cultural Australia

Australia is the *sine que non* of a multi-cultural society. Initial immigration was mainly Anglo-celtic, and the Anglo-celtic heritage and institutions still dominate the culture of the country. In those early days, Australians were not only constituted through penal sentences from the motherland for trivial crimes such as stealing a loaf of bread, but also because of social disruption due to clearances of peasants and crofters from their meagre living footholds in Scotland, and because of impending starvation during the potato famines in Ireland. Population levels were still too low though, to sustain an economy for the support of such a large country with its associated infrastructural needs.

For more than a century, Australia "lived off the sheep's back", with a dominant portion of its wealth coming from the fine wool produced by graziers. As synthetic textiles took over, reliance on wool was evidently dangerous, and diversification

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became imperative. Following on from a gold rush in the mid 1800s, mining of energy and minerals developed strongly and still remains the dominant export earner for the country.

For more than 50 years, Australia has recognised its need to increase its population, given its vast size and small population base. Most of the population is located around the coastal regions of the east coast, south east and south west of the continent. Skills to develop the country have always been short, leading successive governments to encourage immigration of applicants who have skill sets lacking in the Australian workforce. Historically, this has been focussed on tradespeople and construction workers; such immigrants have therefore been the backbone of the current boom in development in Australia. A landmark for this was the construction of the Snowy Mountains Hydroelectric Scheme, which was almost entirely constructed on the basis of jobs provided for immigrant labour, and now represents one of the largest and most powerful clean energy facilities in the world. The scale of this immigration can be judged when one considers that Melbourne, for example, is the third largest Greek city in the world. And yet, Greeks are not the most numerous of immigrants in Melbourne. Italians are even more numerous. Other southern Mediterranean and central and Eastern European immigrants abound in the mix, notably from Turkey.

More recently, immigration from a range of countries has arisen from requests for political or economic asylum. So, now there is a new generation of immigrants from the Adriatic sea region, as well as from south east Asia, notably Vietnam. Immigrants from the Horn of Africa and Persian Gulf are beginning to emerge, and will likely continue and add to the rich multi-cultural mix that is Australia. Each wave of immigrants results in a new generation that identifies culturally both with their (or their parents') country of origin and Australia. A total of (23%) of Australians are born or have a parent born in another country (Fig. 30.1) [1].

All this is to set the background for the variety of cultures, languages and ethnic backgrounds with which genetic counselling services deal on a daily basis. English is not the first language of many Australians and therefore services and information need to be delivered in a large range of languages. Further, culturally influenced lay beliefs about disease causation can affect uptake and influences of the genetic services [2, 3]. Access to Australians where English is not the primary language (or even other language) spoken at home remains a challenge to the delivery of public health services including in genetics.

No chapter on culture of Australia would be complete without attention to the indigenous population. Indigenous Australians are a minority population, but their place in society is increasingly being recognised through land rights, and a popular, though now political, apology for the way they were treated by earlier generations. The latter included the forceful displacement of aboriginal children into foster and institutional care, to ensure their "safety", health and education, but at the expense of the retention of their rich "dreamtime" culture. This has become to be known as "The Stolen Generation". Indigenous Australians live in remote and very remote Australia, but there are also substantial numbers in inner cities, particularly Sydney, and in regional centres. Indigenous Australians have, of course, all the rights, responsibilities and access to services available to any Australian citizen, including healthcare. However, the western healthcare model does not necessarily match its

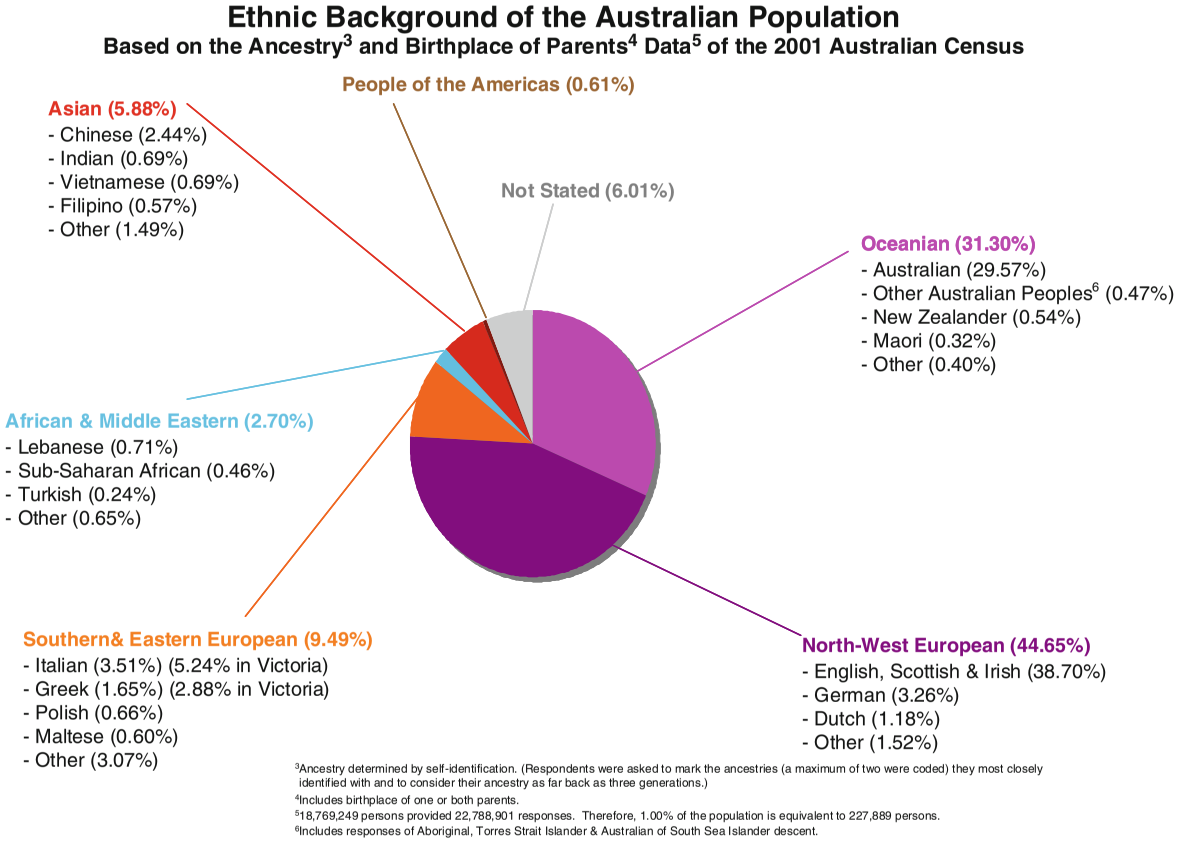

Fig. 30.1 Ethnic background of the Australian population

own expectations and cultural nuances, so there are sometimes delays or blocks to the receipt of what non-indigenous Australians would consider appropriate care. Like many indigenous populations around the world, their health needs are dominated by infections, sometimes alcohol abuse, and increasingly the chronic lifestyle diseases of the west for which they seem less well genetically equipped. Colorectal cancer is not well documented as an important illness for them, but it is likely to rise in incidence. To date, the focus of healthcare for indigenous Australians has been on response to immediate health needs and familial cancer and its management has not been a high priority.

30.2 The Australian Healthcare System

30.2.1 Universal Health Cover

Australia has one of the few genuinely universal health services in the world. All Australian citizens are covered by Medicare Australia, which is partially funded by the Australian (central) Government for all private outpatient services. Inpatient services are available for all Australians, regardless of means, through the public hospital system if they so choose, and as outpatients in a public setting, free of charge. Some services are largely only available through the public health system; among them is access to the DNA mutational analytic services for cancer predisposition.

30.2.2 Public

Most of the genetic counselling services are available only through the public hospitals, where they integrate with the broad range of disciplines, and laboratory support facilities. Public hospitals are funded by state governments, rather than the central government. Thus there is variation across the states with respect to the provision and organisation of services. Interaction across states within a service is not, as a result, seamless, introducing some difficulties in genetic counselling where families are spread across the country.

The nation's public hospitals are the most prestigious, comprehensive, and best equipped in the country. Public hospital appointments are regarded highly by doctors and health professionals, because of their heritage (at least for the older established hospitals), their facilities, and the opportunity for professional exchange and learning. Most are university affiliated, offering also academic backing and input, and fertile opportunities for intellectual exchange with students of many disciplines and educational experience. Governance is independent of universities, reflecting the slightly different organisational missions. Important among the facilities in public hospitals are interpreter services to address the needs of the diverse cultures and languages inherent now in Australia. Such services are much less available in private settings.

30.2.3 Private

The healthcare system is also supported by a strong private sector. Australians can choose to take out health insurance, which will reimburse fees and charges for non-medical care in private hospitals when an inpatient. Medical care in hospital is still largely funded by the universally available Medicare Australia, although there is usually a gap payable to the doctor of a variable amount, part of which may also be picked up through a private insurer. The bulk of the non-medical costs associated with hospitalisation are then claimed from the patient's private insurer.

Private care offers more controllable and ready access to healthcare, especially for elective procedures, and, importantly, choice of doctor. A range of auxiliary services are also reimbursed through private insurers, but not, to date, genetic counselling.

Private hospitals are gaining respect for their range of services offered, and the quality of their services. Most major private hospitals will be equipped with intensive care units and some have emergency departments. Complex surgery can often be handled through such hospitals.

One of the larger private hospitals in Melbourne offers a cancer genetic counselling service, which has independent access to the state's DNA diagnostic laboratory services.

30.2.4 Comprehensive Cancer Centres

Many Australian medical centres aspire to the concept of a Comprehensive Cancer Centre, in the same mould as such centres in the United States. Such centres should have divisions which cover research and prevention as well as treatment, and palliative care. As most of the community programmes on prevention of cancer are developed and implemented through the state cancer councils, or state public health departments, there are few such truly comprehensive cancer centres in the country. Familial cancer clinics and associated genetic counselling should be an important part of such a comprehensive cancer centre.

30.3 Population Screening for Colorectal Cancer in Australia

The Australian Government announced its intention to introduce a population-based screening programme for colorectal cancer in 2002. This followed advice from the Australian Health and Technology Advisory Committee, which first signalled the need in 1997. The advice followed the publication of level 1 evidence that screening for faecal occult blood reduces the death rate from colorectal cancer. A Pilot Programme of screening occurred from 2002 to 2004, and the commencement of full population screening began in August 2006. The initial roll out is to all

Australians turning 55 or 65 years, with an offer of a test, through the mail, every 2 years. The intention is to offer screening to all Australians biennially, from 55 to 75 years of age, through a progressive roll out over the following 10 years. The roll out has been planned to allow the healthcare system to adjust to the demands placed on it through the follow-up of positive tests.

This programme is relevant to the familial colorectal cancer, as the scale of the programme will inevitably increase the awareness of colorectal cancer and its prominence as a health consideration for Australians. Part of the information that accompanies the invitation, is to alert Australians to consider their family history of colorectal cancer, because of the increased risks associated with a family history, and the medical advice to engage in screening through colonoscopy if there are particular familial risk factors. In addition, there is a general practitioner awareness programme accompanying the introduction of screening which focuses on the need to follow up a positive faecal occult blood test with colonoscopy, and also the availability of familial cancer clinics across the country to assess risk and consider the offer of DNA analysis for a mutation in a cancer predisposing gene. Thus, it is anticipated that there will be a more comprehensive ascertainment of families where such a gene may be mutated in the family, and of course an accompanying increased workload for the clinics.

In addition to the impact of population-based screening, clinicians are encouraged to submit tumours resected from all patients under 50 years for analysis of mismatch repair deficiency by clinical practice guidelines from the Australian National Health and Medical Research Council, the Australian Cancer Network and the state cancer councils. This has already been shown to be a powerful means of identifying carriers of mismatch repair mutations and their families in the population [4]. Recently, such an approach has been floated as a standard of care for pathologists to implement, regardless of consent from the patient. This policy is already implemented throughout Western Australia, and is likely to be implemented throughout the country after appropriate ethical consideration in various jurisdictions.

In general, however, a collection of a family history by general practitioners or specialists is the main screening tool by which families with hereditary colorectal cancer are identified.

30.4 Cancer Registries in Australia

30.4.1 Compulsory Registration

Australia has compulsory registration of all cancers throughout the country, as a state function. Registers commenced at different times, and compulsion was also introduced at different times. However, all are now aligned with reporting standards and ontologies, making nation-wide reporting of incidence and mortality possible. Notable among the data emerging is the steady rise in incidence of colorectal cancer over time. Mortality, however, has dropped slightly [1, 5]. An important complement

to the reporting of cancer has been the recent introduction, in some states, of a requirement to report on familial adenomatous polyposis when associated with colorectal cancer [5]. This will also improve the capacity of the healthcare system to identify and direct such individuals to appropriate familial cancer clinics and genetic counselling services.

The state registers publish annual reports reporting in age and population standardised formats. These reports identify trends that need detailed exploration as to cause and consequence and underpin public health planning in cancer and related fields.

Mandatory reporting is countered by strict limitations and governance relating to disclosure of information, especially in any identifiable format. That is, it requires special permission, considered carefully by the cancer councils' or custodians' ethics committees, for the release of any information in an identifiable format. Approaches to individuals on the register for research and clinical management purposes are usually made in the first instance by the Registry to obtain consent to convey details to the investigators of ethically approved research projects. In this way, the register was used to identify all patients presenting under 50 years of age with colorectal cancer, for the purposes of their engagement, with associated consent, in the Australasian Colorectal Cancer Family Study. This has lead to the important insight that the penetrance of mismatch repair mutations ascertained through a population-based sample is not nearly as high as earlier estimates which were derived through studies of families ascertained through familial cancer clinics in Australia [6]. It has also demonstrated that the risk attributable to mutation status is largely up to 55 years of age, as the cumulative risk beyond this can almost entirely be accounted by the normal age-related increase in colorectal cancer incidence seen in the general community [6].

The clinical utility of compulsory registration includes the ability to match surveillance and screening participation with information on cancer development. This will be particularly important in the evaluation of the National Bowel Cancer Screening Programme as it becomes more mature, to provide data on the sensitivity of testing, through registration of interval cancers in the populations accepting screening and returning a negative result.

30.4.2 Special Consideration for Familial Cancer Clinics

In Victoria, the Familial Cancer Clinics have special access to the diagnostic information housed in the Cancer Registries. Ethical consent has been secured to match individuals identifiable on pedigree information provided by probands, with Cancer Registry data. Thus the family tree available at the time of the initial face-to-face consultation at the genetic counselling clinic has verified information on cancer diagnosis, overcoming errors of commission and omission in the proband-ascertained pedigree [7]. This reduces inaccurate risk assessment and inappropriate use of DNA diagnostics and surveillance. A caveat under which the clinics work is that

this verified information, if discrepant from that disclosed by the proband, must not be disclosed to the family although it can be used to perform a risk assessment. In some clinics, this leads to two (colour coded) pedigrees available to the clinical team, one as ascertained by the proband, and the other after cancer registry verification. It is often difficult to explain the ethical issue relating to the clinicians' inability to disclose the information to the proband, as generally there is the reasonable supposition that the information should be shared completely within the family. However, family members consulting the clinics are made aware of this prior to verification and are required to give their consent to this caveat before verification can proceed. Information ascertained directly from other relatives might also be included on pedigrees as the broader family becomes more engaged in the clinic, which needs to be handled sensitively and an ascertainment made by enquiry through the consulting family member as to his/her understanding of the nature of cancers across the family. Not infrequently, especially as the clinic cascades its approach across a family, a wealth of information is gathered across the extended family, through which a clear dominant or recessive pattern of inheritance is evident, but which may be poorly understood by a particular family member due to his/her much less informed knowledge of the cancer status of members across the family [8]. Thus it can happen that screening and surveillance recommendations are made which do not appear to the individual to carry the same evidence base that is apparent to the clinician and counsellors. This takes some careful counselling to achieve the best outcome, both from the point of view of acceptance of the offer for mutational analysis, and more particularly, compliance with recommended screening and surveillance planning for bowel and other syndromic cancers. Of course, the identification of a family specific mutation, and the subsequent availability of predictive DNA testing within the family, eases the dilemma, as the presence of a mutation reinforces the need for compliance, and the absence of mutation dismisses the need for screening.

30.5 Legal Context: Privacy and Confidentiality

Balancing the public institutions that are the Cancer Registries, there are legal and ethical constraints in familial practice, including familial cancer [9].

30.5.1 Family History Information in Clinical Practice

Privacy and confidentiality are important elements in Australian society, and are enshrined in the Australian Privacy Principles [10, 11]. These have legal force. Additional state legislation dictates the collection and use of medical and health information, with considerable penalties for healthcare workers who breach its requirements. Indeed, conflicts over privacy often are highlighted between health professionals and government or legal processes, by professional groups such as the

Australian Medical Association, and recently as it relates to screening for inheritable colorectal cancer [12].

The Australian Privacy Principles [10, 11] impact on the activities of familial cancer clinics in their operations to inform families and provide individuals with options. These laws cover both the collection and use of information. In order to collect health information, the individual concerned must provide their consent. Individuals are deemed to have a right to privacy for 30 years after their death and therefore consent must also be sought to obtain records on a deceased individual; however, it is not entirely clear if the person who may provide consent is the executor of the will – rarely feasible to obtain – or the next of kin. In general, information cannot be collected from one individual about another, but a Public Interest Determination has been made to allow normal family history data to be collected and used, as ascertained through a patient, or in the case of familial cancer clinics, a proband attending the clinic [10]. Thus it is quite legitimate to collect such information for the purposes of managing the proband. The degree to which information about other family members can be used to inform the process of counselling families provides greater challenges for the clinics. For example, a family member may provide information which he/she may not necessarily want disclosed across the family but which may affect risk assessment or the management or other individuals. One way to cope with this is to introduce a consent statement at the time of ascertaining diagnostic information from individuals, to allow clinics to use that information without person-specific disclosure (where possible) across the family for the purposes of counselling, risk assessment and managing the family's risk. In fact, the vast majority of families and their members are quite comfortable disclosing such information for that purpose, so there is rarely a problem. Nevertheless, it is the exception (to such consent) that dictates the modus operandi for all encounters in clinical practice.

There have been multiple community debates in the clinical and genetic community over this issue over recent years, but the law has still not been amended. After much public consultation, the Australian Law Reform Commission recommended a change in legislature with respect to the Public Interest Determination, from allowing disclosure if a threat is serious AND imminent to simply a "serious threat", not necessarily imminent. This would then allow clinics to disclose important genetic information such as the high risk for colorectal cancer implicit for carriers of an APC mutation for familial adenomatous polyposis, directly to family members at risk and, at the same time, an offer of predictive testing and clinical management advice to minimise that risk (for example, a colectomy in an individual affected with familial adenomatous polyposis).

30.5.2 Variation to Constraints on Disclosure

Sometimes, the interests of the community outweigh the right of the individual to complete confidentiality. In the case of some infectious diseases, there is a duty for laboratories to disclose positive findings, relating to, for example, the identification

of a range of infectious diseases. Gastroenteritis due to salmonella food poisoning or legionnaire's disease are such examples, where the states' public health departments will then intervene to try to identify the source of an outbreak, be it a restaurant or cooling tower, respectively. Similar principles may be applicable in the community interest in cancer genetics [12]. As noted above, the relevant Public Interest Determination that allows disclosure of confidential information about a particular patient for the benefit of the community, states that the risk of non-disclosure must be a "serious and imminent threat" to others in the community. While there can be little doubt that an epidemic of salmonella food poisoning represents such a serious and imminent threat, the meaning of these terms with respect to genetically based risk is less clear.

30.5.3 The Power of Consent

Many of the issues mentioned above can be handled effectively by gaining consent for a variety of specified purposes at the time initial consent is secured for diagnostic DNA mutational analysis and this is the approach adopted by familial cancer centres. As well as consent for testing, the proband is asked to give consent for information to be used to provide the opportunity to inform other family members that genetic testing for a cancer predisposing gene is available (without disclosing the individual family member in whom the mutation was identified). He/she can also consent to the information being disclosed to a range of other groups including: the doctor and healthcare professionals caring for him/herself as well as other family members; a state-based familial cancer registry; research and/or projects investigating colorectal cancer and associated genetic syndromes in which the individual is already a consented participant.

Familial cancer is a rich area for research as there are many unresolved questions of familial risk, even after the current genes have been explored in clinical practice. It behoves centres therefore to consider even in the initial consent, the possibility that the family may be usefully engaged in contemporary or future research activities. This can be incorporated in the same consent form. One such consent form that we use is attached (APPENDIX).

In summary, many disclosure issues are easily managed with appropriate consent procedures up front; the power of consent in the face of institutional and community scrutiny of procedures in routine clinical practice is overriding.

30.5.4 Consent and Research

Mention has already been made of consent issues relating to research applications in familial cancer clinics. Indeed, human research ethics committees have taken a leading role in clarifying and supporting approaches to familial studies, now common

in major research-oriented clinical institutions due to the explosion of technologies allowing rapid throughput screening for mutations, and for single nucleotide polymorphisms as markers of association across the human genome. This has greatly facilitated the field, and allowed Australian familial cancer research to advance more effectively than in the more constrained privacy environment of the United States, and in some European countries like Switzerland.

One example of this is the US National Institutes of Health Colon Cancer Family Study. In this large project, population ascertainment of colorectal cancer cases has been well supported, and included in the protocol has been mutational analysis for cancer predisposing genes in appropriately selected individuals, usually on the basis of evidence of mismatch repair deficiency in their tumours. However, the privacy and health insurance implications of disclosure of the mutational information to the individuals was recognised as an issue at recruitment, and so consent for engagement in the project for US participants specifically stated that any DNA risk information would not be disclosed to participants. The reason for this can be understood: such disclosure to the participant of a mutation could easily make that person uninsurable within the American private healthcare system which, for many Americans, is their only access to healthcare. However, the consequences are that the individual is denied access to information which could be life saving and certainly highly relevant to surveillance and screening advice, let alone to other family members who may or may not have engaged in the original research.

Importantly, such non-disclosure disallows the opportunity for these individuals to engage in other important research opportunities, for example, randomised controlled trials of chemoprevention to further the understanding of the means to prevent cancer.

30.6 Genetic and Genetic Counselling Services in Australia

30.6.1 State Variations

All states in Australia have a system of cancer genetic counselling available, which is universally state funded and available to all Australian citizens. Arrangements and governance for this varies between the states. In most states, there is a hub and spoke system, whereby administration of services for the total state is centralised and centrally funded directly from the state.

Tasmania is an interesting example. Tasmania is serviced from the Victorian hub, under contract from the Tasmanian government. With a population of fewer than 500,000 which is relatively immobile, genetic disease is renowned, and competes with other isolated populations such as Newfoundland and Iceland for genetic research interest. Currently, there is intense interest in exploring the opportunities for research into potentially recessive disorders well recognised in the Tasmanian population, and for which no gene has yet been cloned. Hyperplastic polyposis is one such condition.

In New South Wales, cancer genetics is decentralised and funded through interested hospitals, or regional health services. Employment then is through the peripheral organisation. The effectiveness of this approach, on a state-wide basis, is less certain and less easily monitored than the centralised service model. Nevertheless, it does potentially allow for a more effective integration of cancer genetic services into the fabric of those hospitals in which it operates. Where supported by strong within- region or hospital DNA diagnostic services, the model is particularly effective.

Western Australia has a hub and spoke service, with much of the activity at the hub. The Western Australian service has been particularly proactive in applying colorectal cancer genetics as a population-based service, with agreement to test all tumours in patients presenting with earlier age onset colorectal cancer by microsatellite instability testing, backed with immunohistochemistry for the mismatch repair proteins [13]. The result has been the ascertainment of many more families than in the remaining states that depend on referral through other, less comprehensive, strategies. Issues relating to consent have been resolved to their satisfaction in Western Australia, well in advance of the same issues in the eastern states [12].

In Victoria, there are four dedicated familial cancer clinics, and a network of regional genetic counselling services, which meet the needs of rural and metropolitan populations. In general, the genetic counsellors and medical geneticists are funded through the central organisation, Genetic Health Services Victoria, though this arrangement is under review. Organ specialists and medical oncologists are funded through the host institution, in all cases a major hospital. Victoria's centralised governance system is supported by a dedicated software management programme that has functions in clinic intake management including phone call content documentation, clinic planning, computer-assisted pedigree construction, cancer registry verification interaction, support for correspondence which is especially useful where there are signatures required from multiple disciplines, data collection on clinic activities, tumour testing and mutational information, and reporting functions to monitor overall activity in all these areas. This allows budgets to be more easily justified. As it is a state-wide system, there is capacity to link information across clinics and reduce unnecessary overlap in costly processes such as DNA mutational analysis.

Most services offer multi-disciplinary care, typically involving a medical geneticist, genetic counsellors, organ specialist, and medical oncologist. Mostly, these clinics are truly multi-disciplinary, with most of the disciplines attending at the one clinic at the same time. However, in some states, there is a disconnect between the core genetic services of risk assessment and DNA mutational analysis and the clinical management that follows from that advice. This leads to difficulties in monitoring clinical outcomes from the service and introduces another step for communication breakdown between health professionals and families. Despite this, clinics operating with this mode have been very effective, especially in the smaller states.

In the multi-disciplinary clinics, divisions of responsibility usually fall logically, with the genetic counsellors taking the bulk of organisational responsibilities and day to day management of the clinics and their appointments. In our own clinic,

there is a close linkage with our much more long-standing Bowel Cancer Surveillance Service, which also assesses risk (informed often but not always from our familial cancer clinic), plans surveillance against a template of national guidelines, and contacts patients at pre-defined intervals to arrange or prompt their attendance for surveillance procedures [14].

30.6.2 Benefits of Centralization

The benefits of a centralised service are clear from the above description relating to Victoria. It is especially evident in genetic services, where the unit of consultation is much more the family than the individual, when compared to the usual modes of clinical practice. Families are usually spread geographically, so a state-based system is important. Of course, some families have member's interstate. However, whereas Tasmania is the archetype of an immobile population, the same holds generally true for the majority of the Australian population. Unlike Americans, who generally have difficulty with the question "Where do you come from?" Australians can readily answer the question as their family roots are usually well secured, at least within a state. This adds to the ease and utility of a state-based familial cancer clinic service.

Additional benefits come from a close monitoring of activity across the state, a greater capacity to allocate limited personnel resources to areas of need, a greater *esprit de corps* within the genetic services community, and substantial advantages in teaching and opportunities for professional continuing medical education and genetic research. Interaction with interstate services, particularly where they too are centralised, can be streamlined, including the collation of national data. National meetings can be focussed quickly on emerging clinical developmental and research areas of interest. Finally, international collaboration in projects that require access to larger populations is more easily feasible. The Australian contribution to the international CAPP2 study of resistant starch and aspirin in mismatch repair gene carriers is an outstanding example of this. Australia contributed 12% of the recruits to this project involving over 1,000 persons, a proportion much greater than our overall population compared to that of the western world.

30.6.3 Integration with Adult Hospitals

There are some limitations to the centralised model. Major hospitals do not, in the main, enjoy being directed as to the use and deployment of their facilities by outside institutions. This is particularly true where the major hospital is an adult hospital. Historically, most genetic services in Australia have emerged from a paediatric home of excellence. The needs of adult hospitals do not always align with a paediatric

focus, which is ever more clear as the science of genetics penetrates with ever more relevance across a range of diseases only expressed in adults, and as a result, tensions in competing service delivery needs can emerge. Further, growing demand for genetic expertise has not been matched by the availability of appropriately trained personnel, and a shortage of personnel can compromise the best laid plans in decentralised service models.

30.6.4 Training

As is implied by the above, a range of health professionals provide services in colorectal cancer genetics services. As well as medical specialists in gastroenterology, surgery and clinical genetics, genetic counsellors are an integral component of services. Genetic counsellors in Australia have usually completed a post-graduate training programme in genetic counselling and a certification process through the Human Genetics Society of Australasia Board of Censors (genetic counselling). They commonly have a professional background in nursing, science, teaching, psychology, social work, or other related area. Nurses have some involvement in clinics in some centres, usually related to aspects of management of colorectal surveillance. The term "genetic nurse" does not have the same use in Australia as in the USA or UK, as the majority of nursing professionals practising in genetics are considered genetic counsellors.

The teaching programme is fundamentally clinic based with graded responsibilities for students. The curriculum is sound. Medical geneticists also train through the same service, qualifying with recognition from the Royal Australasian College of Physicians.

30.6.5 Research

Genetic counselling is essentially a clinical service, and counsellors as they graduate have a clinical focus to their work. However, the explosion of interest in genetics inevitably engages the clinics, as the patient resources for research are best ascertained through the familial cancer clinics. The clinics are indeed a fabulous environment for clinical research, especially in translation of the many genetic discoveries in cancer predisposition, as well as the recognition of family phenotypes very likely to be genetically determined, but for which no genes have yet been discovered. Further, research in surveillance including outcome benefits can be focussed through the clinics and their associated surveillance services, including the evaluation of new screening modalities such as MRI scanning. Close liaison with DNA diagnostic laboratories, which frequently have research activities associated with them at the laboratory level, allows an

interaction with scientists to focus their attentions on important questions that emerge in clinical practice.

30.7 Clinical Approach to Counselling in Australia

At the outset, it must be made clear that there are fundamental differences in the approach to families and their members, where there is, as yet, no germline mutation identified, as opposed to individuals in families where there is a germline family specific mutation identified, but the particular family member in consultation has, as yet, not been tested for this mutation. In the former case, there is a need for a full evaluation of the gene *(mutational analysis)*, informed usually in the case of HNPCC, by immunohistochemical findings of a particular protein loss in an affected member of the family (which is usually the person also offered mutational analysis). In the latter case, *predictive DNA testing* exclusively focuses in a most cost-effective fashion only on the particular family specific mutation identified in another affected member of the family who may be more or less remotely positioned to the consulting family member on the pedigree. The process described below focuses on that undertaken when a germline mutation has not been identified.

30.7.1 Referrals

Referrals to most clinics are relatively unconstrained. A simple telephone call (self-referral) to one of the nation's clinics from a family member would be enough to set in train a process which eventually may lead to the identification of a family specific mutation in a cancer predisposing gene. Alternate modes of referral are from other family members, cascade approaches through the family, interstate and international services and registries, general practitioners, surgeons, physicians including medical oncologists, pathologists, research investigators investigating population incidences of familial cancer, and in some states, routine testing of early onset cancers for evidence of mismatch repair deficiency. These processes have recently been reviewed [15].

30.7.2 Intake

Genetic counsellors are responsible for accepting and responding to the various modes of referral. As much relevant information as can be ascertained is secured over the phone and documented in clinic records, which is computerised in some centres. A focus on the number, and ages of onset, of syndrome-related cancers is made, and an assessment as to the likely benefit of clinic engagement. This is used

to perform a preliminary risk assessment, which determines the subsequent handling of the referral. Clinics across the country focus principally on either just high-risk families as measured against the National Health and Medical Research Council Guidelines, or moderate and high-risk families [16]. A questionnaire is then sent to the applicants whose family history suggests a sufficient risk for a genetic susceptibility to colorectal cancer.

30.7.3 Family History Ascertainment

The questionnaire, structured to collect information on familial risk, is returned to the clinic. This includes identifying information on relatives, both affected and unaffected, and their perceived cancer status by organ, their relationship to the proband, and the age of onset of the cancer and, if relevant, date of death, as well as any medical personnel and the hospitals known to have attended to a family member with cancer.

30.7.4 Family Tree Construction and Verification of Diagnoses

An electronic family tree is constructed from the unverified information on the returned questionnaire and a matching process with the state cancer registry may be undertaken. There are no unique identifiers for Australians, so computer matching algorithms are needed to enable the closest match possible. Verified diagnoses emerge, at least as far back as the cancer registries record cancer diagnoses (generally approximately 30 years). With appropriate consents, attempts to obtain colonoscopy and pathology records are made in advance to improve efficiencies in risk assessment and the later consultative process. A second verified family tree is constructed with the additional information availed. The verification process adds immensely to the confidence with which the risk assessment process can proceed for an individual family and subsequent diagnostic and management options available to the individual can be made. The confidentiality issues abounding around the process have been discussed above.

30.7.5 Multi-disciplinary Meeting

Multi-disciplinary meetings are held to discuss each family, identifying gaps in knowledge about the family, and making a preliminary assessment of risk and availability of mutational analysis to the family members. Risk assessment will generally be informed by guidelines such as the Bethesda criteria [17] and modified Amsterdam criteria [18] for hereditary non-polyposis colorectal cancer, or evaluation of risk of polyposis syndromes including all information from colonoscopies

and associated pathology relating to the number and type of polyps removed through the patient's entire colonoscopic or surgical history. Based on the likelihood that the family history is due to a known genetic syndrome and the availability of a family member in whom tumour testing for evidence of mismatch repair deficiency (in the case of HNPCC) and/or germline testing may be undertaken, a tentative plan is made. Strategies of surveillance for the consulting family member and the family as a whole are provisionally decided. Finally, any open research opportunities for which the family and their circumstances may be eligible are considered.

30.7.6 The First Consultation

30.7.6.1 Checking the Pedigree

After introduction, and an enquiry about what the consulting family member hopes to achieve from engagement in the clinic, the pedigree information is checked as per the patient derived pedigree and an attempt to fill out important gaps of information relevant to syndromic recognition highlighted; strategies to secure further relevant information are discussed.

30.7.6.2 Risk Assessment and Tumour Testing

A more mature assessment of risk is then undertaken, based on all available information. A strategy, including its rationale, to determine whether a mismatch repair or polyposis gene mutation is likely is developed, and discussed with the family member. Typically this would be, in the case of suspected hereditary non-polyposis colorectal cancer, an offer to test a key family member's tumour for evidence of mismatch repair deficiency, as discussed below. In many services, immunohistochemical information on mismatch repair protein loss is needed before any attempt to determine a germline mutation is undertaken. This is because it is much cheaper to screen first for evidence of mismatch repair deficiency in tumours, and because germline mutations without mismatch repair deficiency in the tumours are very uncommon indeed. Further, immunohistochemistry can pinpoint which of the four mismatch genes is responsible, obviating three quarters of the mutational analytic search needed without this information. Ideally, the youngest member of the family affected with a syndromic cancer is tested, although specimens archived for over 20 years generally deteriorate to the point where it is not worthwhile pursuing access to them.

As a component of the risk assessment discussion, and often preceding it, the counsellor will explore the individuals pre-existing belief(s) about the cause of their family history of cancer and their own risk. Lay beliefs can influence receptivity to genetic information [19].

30.7.6.3 Syndromic Information

If hereditary non-polyposis is suspected, the family is provided with verbal and written information about the syndrome, including the range of syndromic cancers, typical age of onset, penetrance, inheritance, tumour testing for evidence of mismatch repair deficiency, initial mutational analysis to identify the family specific mutation, accuracy of testing, cancer management, and the benefits of surveillance. A resource for this has been developed by the Cancer Council of Victoria, recently complemented by the book "Colorectal Cancer Can Run in the Family", and edited by Terri Berk and Finlay Macrae [20].

30.7.6.4 Penetrance

The concept of penetrance of the mutation (the chances of developing disease for a mutation carrier) and the differences in estimates of penetrance in clinic-based verus population-based research studies is discussed [6]. The high penetrance reflects on the need to address the risk through surveillance planning.

30.7.6.5 Mode of Inheritance

Information is provided on mode of inheritance for the gene in question in the family, or the likely gene. In all but one case in colorectal cancer genetics, the inheritance is dominant, so simple card or other descriptive techniques are used to transmit understanding of dominant inheritance with its one in two chance of transmission to offspring, and the completion of a line of transmission if an individual in a family does not inherit the mutation … "the line is cut, so to speak". The single current exception, the recessively inherited MYH associated polyposis, may also be explained with its risk to siblings rather than the next generation.

30.7.6.6 Pre-genetic Testing and Mismatch Repair Deficiency

Pre-genetic testing seeking evidence of mismatch repair deficiency in the family is a crucial strategy in the approach. As described above, the youngest syndromic-affected member's tumour will be targeted, and strategies to obtain the consent of that individual, or his/her next of kin if deceased, planned. If it is the proband attending, the process can commence immediately, otherwise the relevant family member or (if deceased) their next of kin is approached by the family member attending at consultation. The rationale for such tumour testing is described elsewhere in this book.

30.7.6.7 Mutational Analysis

If the information is mature, that is, if there is a high likelihood that HNPCC has caused the family history, a search for the family specific mutation, can be discussed in detail with the family member concerned. Typically, it is the youngest member of the family affected with colorectal cancer in whom immunohistochemistry has indicated the likely gene. Cancer predisposition genes are notorious for the diverse location of the mutations across the gene, and make the mutational analytic search difficult. An explanation is provided about this, usually in terms of "looking for a needle in a haystack" where the gene is represented by the haystack, and the needle is the mutation, or by way of locating a street address in a city, perhaps complemented by a spelling mistake analogy in sentence construction. In any case, it is pointed out that the search might take months to even years, and even in the most compelling of family situations, can never be guaranteed to uncover the family specific mutation.

30.7.6.8 Insurance Considerations

Insurance issues are relevant in Australia but are different in nature to countries with a predominantly private health system such as the United States. The first point we make is that for an affected person, knowledge about their gene status adds little if anything to their risk as assessed by their insurers. That is, the cancer itself dominates by far the risk assessment, to the point where genetic predisposition information is unimportant for insurance purposes. So there is no need for an affected individual to feel constrained on account of any personal insurance consideration.

For unaffected family members contemplating predictive DNA analysis, there are, however, some implications [21, 22].

Income, disability, and trauma insurance may be affected: individuals are required to disclose their *own* genetic test results and fully disclose the family history. Failure to disclose this information known at the time of application may make the policy void at the time of a subsequent claim. In general, we counsel family members to review their life and income/trauma insurance before submitting for *predictive* DNA analysis and written information for families is provided.

There is a voluntary agreement among members of the peak industry insurance association (IFSA) that information relating to the genetic status of another family member cannot be used to assess the risk of an applicant. Insurance companies are required to be transparent about the basis of their decisions about refusals to provide cover or to place loadings or restrictions on the policy, and this needs to be based on actuarial data. These issues are explored actively in the community, in great detail and aim to provide a level playing field of risk knowledge between the insurer and applicant thus avoiding higher utilisation of insurance by those at higher risk resulting in what is known as "anti-selection", which is a trend that threatens the viability of the insurance industry. In fact, it appears that genetic

discrimination is an uncommon experience and that there is variability in response to family history among insurers [22]. Importantly, in Australia, health insurance is not relevant in the discussion. This is because health insurance in Australia is "community rated", meaning that private insurers are legally not allowed to discriminate between applicants based on their health status [21]. They can deny payments for up to 12 months after taking out a policy, but not beyond that, regardless of health, genetic, or any other status. In any case, the universal healthcare system will cover any Australian for public hospital inpatient care, insured or uninsured, at any time.

30.7.6.9 Insurance Considerations

Therefore, the affected proband should feel his actions in agreeing and consenting to the initial mutational analysis, as a family-specific mutation cannot be used to influence the insurability of other members of the family, who may or may not carry such a mutation [23]. Some insurers will request information on whether a relevant mutation has been identified somewhere in the family, but their statement of conduct does not allow them to use this information in risk assessment.

30.7.6.10 Consent

Consent to testing proceeds with all the considerations discussed above. Consent for germline genetic testing is well established and enshrined in the NHMRC guidelines for practice [10]. However, mirroring practice elsewhere [24], an important debate in Australia is the necessity, or otherwise, for consent to perform tumour testing. In general, clinics do obtain such consent, but there is an increasing willingness to consider non-consented tumour testing, especially where there is a population-based approach to tumour testing such as in Western Australia [12]. At present, opinion-leading organisations such as the Hereditary Bowel Cancer Group at the Cancer Council of Victoria are preparing statements to legitimise the approach of non-consented tumour testing, noting that it is a phenotypic test of a tumour, and akin to a range of immunohistochemistry tests that are routinely used by pathologists to assist in the clinical management of a variety of tumours. The hesitation has been because of the relatively tight relationship between the finding of MSH2 loss in a tumour, and a germline mutation in MSH2. However, more extensive studies recently have defined this association at around 80% and not 100%, making even MSH2 loss in a tumour still only a marker for a germline mutation. The implications for a non-consenting policy for tumour testing are important in the context of moving ahead with routine testing for mismatch repair protein loss in a population selected only for young age of onset of colorectal, or endometrial, cancer. It will also allow pathologists to follow their natural inclination to test for mismatch repair deficiency if they identify morphological features suggesting this – tumour infiltrating lymphocytes, mucinous morphology, or Crohnoid reactions.

30.7.6.11 Benefits of Surveillance

Surveillance is the response that the healthcare system can offer carriers of mutations, or those with a relatively high risk, to protect them from the development of a lethal cancer. Evidence from Finland underpins the veracity of this statement, albeit from a non-randomised but still controlled trial [25]. In the Finnish experience, the benefits of surveillance were clear and meaningful. Equally, family members testing negative for the family-specific mutation can be reassured that their risk is no greater than the general population, and any previous surveillance strategies relating to their risk assessed on family history can be downgraded to population-based screening recommendations for average risk persons – provided there are no other risk factors (such as previous sporadic adenomas) present.

30.7.6.12 Surveillance Recommendations and Planning

Assimilating all the risk information, a surveillance plan will be discussed and recommended for the consulting family member and the entire family. This may be modified as more information becomes available, particularly with respect to the mutation detection. National Health and Medical Research Council Guidelines for surveillance on the basis of family history and/or mutation status are used, covering most familial situations, and closely aligned to international guidelines. An exception is the recommendation that screening first-degree relatives in families is restricted to families at moderate risk; families with only a single first-degree relative with colorectal cancer at an older age of onset do not attract screening recommendation involving colonoscopy [10].

30.7.6.13 Primary Prevention Advice

Advice on primary prevention is provided in our clinic. This is founded on the Australian Polyp Prevention Project [26], whose results are also reflected in national dietary guidelines for colorectal cancer prevention. In summary, these are

- To reduce dietary fat to 25% of calories, by selecting low fat cuts of meat, trimming off all excess fat from meat, using low-fat diary products, avoiding junk foods and spreads
- To include plenty of cruciferous vegetables in the diet
- To include half to a third of unprocessed wheat bran daily
- To avoid alcohol, especially beer

30.7.6.14 Research

Research opportunities are an integral part of the consultation and planned in advance. A range of research projects addressing the needs of families and clinics is always operational in many clinic activities (see below).

30.7.6.15 The Clinic Correspondence

A clear departure from normal clinical practice in surgery and medicine is the policy of writing directly to the consulting family members after the clinic, summarising the sometimes complex information disclosed and discussed at the consultation. This is a very valuable part of the process and one that is valued by families. In one Australian study, the receipt of a post-clinic letter was significantly associated with knowledge about the relevant hereditary cancer [27]. Letters also go to the other doctors caring for the family member, which at times might only be a cover note accompanying the main epistle sent to the family member. In our clinic, the management of this correspondence, which often will involve multiple disciplines through their signatures and letter construction, is computer assisted, passing correspondence between team members with a final sign off before printing and mailing.

30.7.7 The Second Consultation

A second consultation is offered to family members considering genetic testing. In practice, the initial affected proband who is offered mutational analysis for the family-specific mutation is often attending the clinic with a desire to undergo genetic testing and request that blood be drawn at the first appointment. Those undergoing predictive DNA testing are encouraged to take time before venesection to integrate the large amount of information they are given and consider the impact of possible results.

This can also be an opportunity to collect further information if available about the family.

30.7.8 The Third Consultation: Disclosure of Mutation

Once the mutational status of the individual has been clarified, the proband or other family member is invited to a third clinic attendance, where the result is disclosed. For the initial mutational search in the proband, this may be many months or years after the DNA was collected. So, a review of the family history is in order. If the DNA result is uninformative (no mutation identified), the likelihood of a genetic predisposition to cancer is reviewed. If appropriate, alternate testing strategies might be discussed. This could include testing other family members, or testing the same family member with newer and different techniques such as multiple ligand probe analysis (MLPA), looking for large deletions or rearrangements in the gene which could be missed with techniques requiring preliminary amplification of the DNA because the deletion may involve a key primer site, essential to the

amplification process. The implications of the predictive DNA results in family members unaffected with cancer are reinforced – either intensification of screening if the result is positive, or reduction in surveillance to average risk population screening if negative.

Implications for children of carriers are usually foremost in the minds of family members at this stage. The 50% risk to children and siblings for transmission of the affected allele (in dominantly inherited syndromes) and the risk to siblings (in recessively inherited MYH associated polyposis) are again covered. The lack of any risk for the family syndrome of cancer for the children (but not the siblings) is highlighted where the result is negative.

Increasingly, the technologies associated with reproduction bear discussion in young family members. Already there have been some families opting for pre-implantation genetic diagnosis in familial adenomatosis polyposis, which is supported by the Australian healthcare system.

30.7.9 Disclosure of Family Specific Mutational Information to Other Family Members

The legal context in which discussions about transmission of information about risk, testing, and surveillance occur has been discussed above. In the clinic, the intention of the consulting family member to inform other family members is explored and the counsellor attempts to prepare for possible responses. As found elsewhere, it is rare for an individual to refuse to inform family members [28]. However, it is clear that transmission of information can be incomplete, leaving some or many family members exposed to a risk that they could control through appropriate predictive DNA testing [29, 30]. This "passive non-disclosure" appears when there is little opportunity or responsibility felt to pass on the information across the family. The techniques to handle this situation in Australia are evolving and are practical in focus; the commonest is to provide a letter for the proband to send or take to all relevant relatives, which indicates that a mutation has been found in the family which may be of importance to other family members, without specifying who the individual who has been tested actually is. The letter provides information about the location and phone numbers of the state's familial cancer clinics, and sufficient information for the clinics to make the connexion with the relevant clinic which holds the family-specific mutational information. Other branches of the family can be engaged opportunistically as they attend clinics, to increase the likelihood of all at-risk relatives being contacted within normal family communication channels [31]. Active non-disclosure, where the proband specifically forbids clinics to disclose information relating to his/her genetic mutational status to one or more other family members, is a thornier ethical dilemma, even though it can be argued that the information pertains to the family, not just the individual. In this

case, it is important to first explore the source of the reluctance to inform relatives as there may be a desire to protect family members from potentially harmful news [30]. If other relatives are already in contact with the service or a registry, this situation can generally be handled by not disclosing the individual source of the family-specific mutational information, but if transmission relies on the tested individual then this can place the clinical staff in a legally dubious situation, as described above.

In Victoria a new protocol for disclosure of familial adenomatous polyposis risk information is being planned and implemented. Familial adenomatous polyposis represents "the pointy end of the debate" because there is a 100% cancer risk for carriers, and there is effective prophylactic therapy available through colectomy. A policy of direct disclosure to identify at-risk relatives has been developed in consultation with legal counsel. For the less penetrant cancer predispositions including hereditary non-polyposis colorectal cancer, a randomised controlled trial of direct or intra-familial disclosure has commenced within a research setting, with careful gathering of information relating to the responses from the families and the relative effectiveness of the different approaches.

If the Australian Law Reform Commission's recommendation to alter the Public Interest Determination to allow disclosure where there is only a serious (not imminent as well) threat to others becomes enshrined in law, there is concern that there will be an additional onus of responsibility placed on the clinics and familial cancer registries to ensure disclosure across the family, with a concomitant (legal) responsibility for the development of an advanced, potentially avoidable, cancer by a family member who does not appear to have been informed. There is a need to achieve a balance between these concerns and the need to inform family members of their risk, and minimise the lethal consequences of such risk, in the best and most effective way we can. One expects the legal system will protect *bone fide* and organised attempts to do this, for the community's well-being.

30.7.10 Surveillance Services

Surveillance recommendations usually form part of the third consultation. However, the systematic *provision* of surveillance services to support genetic counselling varies between services. Those clinics that work best probably have facilities for surveillance in-house, or at least a service that plans, reminds, and monitors surveillance even if remotely performed. At The Royal Melbourne Hospital there has been a much longer engagement in planning surveillance than in the operation of familial cancer clinics, as the establishment of the service long preceded the cloning of the cancer predisposition genes. The service also offers annual faecal occult blood testing, a service which, prior to the implementation of the National Bowel Cancer Screening Programme, was unavailable to the community – and even now is available only to a selected age band in the community through the national programme. The two services – familial cancer clinic and Bowel Cancer Surveillance Service – work hand in

hand. It is important that surveillance remains under the control and implementation of the referring doctor, especially if that doctor has the relevant skills, for example, in colonoscopy.

30.7.11 Clinical Meetings Supporting Genetic Counselling for Cancer in Australia

30.7.11.1 Hospital Meetings

All familial cancer clinics in Australia would be supported by a structured programme of clinical meetings to explore issues relevant to their clinical needs. These are characterised by a healthy interchange been laboratory scientists and clinicians. Indeed, such interaction between laboratory scientists and clinicians are not only characteristic, but essential for the most effective delivery of care to families attending the clinic. Indeed, it is difficult to identify a laboratory interaction that is both more necessary and more effective than the interaction that occurs around familial cancer. Anatomical pathology may be the exception, which is also important in familial cancer.

Hospital meetings are also important more generally to "fly the flag" for the place of genetics in mainstream disciplines in adult hospitals, which have some risk of being blinkered to the amazing advances in genetics and clinical genetics.

30.7.11.2 State-wide Familial Cancer Clinic Meetings

Meetings that collect the clinicians, including genetic counsellors, from familial cancer clinics across the state are important as they help to standardise clinical approaches to general and specific clinical presentations across the clinics. In a relatively new discipline, there is a risk that clinics may diverge in their approaches to clinical problems. While this may be healthy in general clinical practice, providing a variety of valid approaches to solve problems, it can introduce difficulties in familial practice where one branch of the family may receive opposing advice about, for example, surveillance protocols for at-risk relatives, compared with advice from another. This may devalue the advice from the service as a whole and lead to a paralysis in compliance to any surveillance. If there are differences, then clinics can be informed of the differences and make appropriate explanations to families, most likely based on the foundation being expert opinion, rather than more empirical, and thus firmly based, evidence.

State-wide meetings, if appropriately couched in privacy agreement, also provide opportunity for clinics to share family informational difficulties, and reach the best judgement about the management of a family's particular circumstances.

At another level, state-wide meetings provide the forum for professionals, and indeed consumer representatives, to carve out guidelines for clinical practice based

on scrutiny of the available international literature and guidelines such as those developed through InSiGHT – the International **S**oc**i**ety for **G**astrointestinal **H**ereditary **T**umours.

In our community, these state-wide meetings are hosted through the Hospital neutral territory of the Cancer Council of Victoria.

30.7.11.3 The Cancer Councils

As can be gleaned from the previous commentary, in many states, the non-governmental organisations (NGOs) of the state cancer councils play an important and integral part in the delivery of cancer genetic services in Australia, particularly in Victoria, where that state's cancer council is strong, effective, and highly respected in the community.

Cancer Councils also play a very important role in community awareness of cancer, and the services that are available to the community through state-funded familial cancer clinics. The cancer council's HELPLINES are an institution with high community credibility and serve the constituents well. The advice they deliver is thoughtful, sensitive, and evidence based, with ready referral to medical services in the community. The phone number is readily available.

Cancer Councils also have an important place in lobbying governments for policies and services to protect the community against cancer. Notable among these are smoking restriction and sun protection, but they also direct governmental attention to the benefits and needs of familial cancer clinics in the new age of cancer genetics. Governments are, by their nature, always faced with lobby groups advocating for different causes, and cancer sufferers and their families also need effective representation to government which, in our culture, is often effectively delivered by the state cancer councils, or collectively, a national consortium of cancer councils.

Cancer Councils are also an important source for funding research, as their outreach to the community is accompanied by reciprocal generosity in donations and bequeaths. Indeed, some of the nation's most prestigious and successful cancer researchers have had life-long funding through such support from community donations.

30.7.11.4 Human Genetics Society of Australasia

This professional organisation represents the interests of the medical genetic and genetic counselling community and has a special interest group in cancer genetics. It offers scientific fora for presentation of clinical genetic research, through its annual meetings. It also plays an important role in defining guidelines and standards of care across clinical genetics and contributes to the development of these by other bodies in cancer genetics. It provides grounding advice to health professionals in the areas of practice which are quintessential to the generic conduct of medical genetic and counselling services.

30.7.11.5 Organ and Disease Specialty Meetings

Parallel to this are organ and disease specialty meetings which also have a stake hold through their members who are engaged in the multi-disciplinary delivery of familial cancer clinics. This would include the Clinical Oncological Society of Australia and, as far as colorectal cancer genetics is concerned, the Gastroenterological Society of Australia and the Colorectal Surgical Society of Australia. Gynaecological Colleges also are stakeholders, given the definition of the syndromes across organ specialties. Perhaps, with time, we will see the development of national organisations focussed specifically on hereditary gastrointestinal tumours, reflecting the international organisations of InSiGHT and the Collaborative Group of the Americas – themselves focussed on familial colorectal cancer.

30.7.11.6 Research Organisations

The most effective organisations to date at a national and international level are the research-based organisations. The US National Institutes of Health-funded Australasian Colorectal Cancer Family Register has for more than a decade underpinned the meeting of clinicians and investigators engaged in familial colorectal cancer, as most of the clinics have been part of the mammoth international effort. Funding for such meetings through the research projects has been the key to these meetings, which commonly meet alongside kindred research organisations interested in familial "other" cancers. Often there are similar issues to manage and so the opportunities to interact are fruitful.

Recently the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) has also defined an interest in colorectal cancer prevention, and their wide-ranging expertise across biology, mathematics, and bioinformatics has been drawn to focus on this disease. This has included important projects in cancer genetics, especially in projects to define cancer predisposition at the germline level. This too has enhanced opportunities for exchange of information and ideas across the nation's familial colorectal cancer clinic professionals in an exciting fashion, through meetings and collaborations between the CSIRO and targeted familial colorectal cancer clinics.

30.7.11.7 Research

Little more needs to be said about research as one can see that there is a thread of research that runs through most of the cancer genetic services in the country, funded locally or, because of the organisational expertise in Australian service delivery, from international sources. Research has been deliberately filtered through this chapter, as it should be seen as part of the fabric of clinical care, for the benefit of the contemporary family and its future generations. This has lead to Australians playing prominent roles in the international steering committees of these projects, as the Australian contribution is highly valued. This applies equally as well to

funding and projects emanating from USA as from Europe. Australian clinics stand to benefit and contribute in an exciting fashion from both continents, and are very much in the scale of world activities.

Parallel academic research in genetic counselling is part of the Australian culture, with a succession of research focussing on real clinical issues of disclosure, psychosocial impacts of genetic counselling, and the impact of serious diseases on the resilience and adaptability of families so afflicted. Acceptance of recommendations, consent protocols, and emotional impacts of counselling advice also are under evaluation, including in the vulnerable paediatric age groups.

Interaction with International Organisations and Expertise

Also exciting is the effective interchange with colleagues overseas who are often facing similar challenges. The tyranny of distance is an ever present reality in Australia, making it imperative for healthcare professionals in Australia to travel to international meetings and experience the uncertainties and evidence-based certainties of practice as seen by colleagues around the world. Australian contributions to these meetings are also strong, and add importantly to the bodies of knowledge in the field which eventually may filter into clinical practice here and around the world. At present, notable in this domain is the work from the Garvin Institute in Sydney where new mechanisms or cancer predisposition are being defined through an understanding of epigenetics, which surprisingly, may also be inheritable. New paradigms in thinking to the conventional clinical geneticist have emerged, providing a powerful explanation for some of the anomalies seen in clinical practice where there is somatic evidence of a possible germline mutation, but nothing found after intense molecular scrutiny of the relevant gene often in question. MLH1 and MSH2 are engaging attention worldwide for this inheritable epigenetic mechanism.

The most important interaction for familial bowel cancer clinics in Australia has been with InSiGHT, as it is this organisation, and its predecessors the Leeds Castle Polyposis Group and International Collaborative Group for Hereditary Non Polyposis Colorectal Cancer, which has been so influential in the development of clinical practice in the area, and been a foundation for the expression of research and, where necessary, its international organisation. Nowhere more evident than this has been the huge effort of the CAPP group to implement and complete a trial of aspirin and resistant starch in mismatch repair gene carriers, or their phenotypic counterpart in families expressing all the attributes of the syndrome but without a defined germline mutation.

Engagement in such international activities both informs local practice and contributes to international opinion. It also places Australian investigators in key positions for opinion leadership, which itself helps shape clinical practice. Australians lead the world's thinking in the Human Variome Project, which is now closely aligned with InSiGHT as far as familial colorectal cancer is concerned.

We are indeed a lucky country being able to turn equally to Europe and North America to assess and reap the rewards of each of these continents, as well as importantly contribute in our own way to the global efforts in familial colorectal cancer.

APPENDIX

30.8 Familial Cancer Centre

30.8.1 Consent Form for Genetic Testing

This form has been designed to ensure that consent for testing is on an informed basis. Please read and consider each section.

30.8.1.1 Genetic Testing

I understand that:

- My blood/pathology sample will be used to examine my genetic material and tested for one or more of the genes involved in predisposing to:
- Hereditary breast/ovarian cancer
- Hereditary Non Polyposis Colorectal Cancer (HNPCC)
- Familial Adenomatous Polyposis (FAP)
- Other (specify)
- The testing is completely voluntary and it is possible to withdraw from the testing process at any stage
- Testing may identify genetic changes (mutations). These changes may be present in other members of my family

30.8.1.2 What are the implications of genetic testing?

I understand that:

- Alterations (mutations) in cancer predisposing genes cause a high, but not a certain risk of cancer.
- The test may show the presence of a mutation but it cannot accurately predict the age of onset or type of cancer that may develop as a result.
- The test may not reveal all possible mutations that may occur in the genes tested.
- Test results of one individual can change the estimation of risk for other family members who have not requested testing
- Test results of one individual may affect the ability of family members and/or myself to obtain some types of insurance.

30.8.1.3 What will be done with the test results?

I agree that:

- The test results will be held by The Royal Melbourne Hospital and will be known by those participating in providing the test.
- Information relating to my testing will not be revealed or made available to any other person/organisation, except with my consent (see below) or when disclosure is required by law.
- The results may be used to help the counselling and testing of other family members, provided that to do so would not reveal any details of my identity or personal test result without my consent.

I consent to my test results being made available at any time to the following people:

- Any family member
- Only to the following individuals *(specify)* _____
- My doctor(s) *(specify)* _____
- Research group *(specify)* _____
- No other individual

  In the event of my death, my test results should be released to *(name) (address)*

  _____

30.8.1.4 What will be done with the sample after testing?

I agree that:

- The sample will remain the property of the laboratory. It will be stored in good faith but its suitability for future use cannot be guaranteed.
- The sample may be examined again in the future using new methods.
- My identified sample will not be used for any other purpose except in accordance with my written consent
- It may be disposed of at a time determined by standard laboratory practices or regulatory requirements.

30.8.1.5 Research

After testing has been completed

- I consent to my potentially identifiable sample being used for future RMH Clinical Research and Ethics Committee approved research.

  Or

– My DNA sample may not be used for research without my written consent for that research
  Or
– My DNA sample may not be used for research and I do not wish to be contacted regarding research.

Signature of Individual _____
Date _____
Printed Name _____
Date of Birth
ATTACH BRADMA LABEL

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Chapter 31
Across Culture and Health Systems: Korea

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Abstract Hereditary colorectal cancer in Korea will be described in this chapter. Particular emphasis on the establishment and activities of the Korean Hereditary Tumor Registry will be given. The Korean Health Insurance System including support for genetic testing and the National Cancer Screening Program will also be discussed.

Keywords Across • Culture • Health • System • Hereditary • Colorectal • Cancer • Korea

In Korea, colorectal cancer is the fourth most common malignancy in men after cancer of the lung, stomach, and liver, and the third most common in women after stomach and lung cancer. The crude incidence rates per year are 24.2 and 19.6 cases per 100,000 Korean males and females, respectively [1]. Among 99,025 new patients recorded by the Korea Central Cancer Registry in 2002, 11,097 cases involved colorectal cancers, accounting for 11.2% of all malignancies. A comparison of the annual incidence of colorectal cancers in 2002 to that in 1995 revealed an increase of 184% in men and 164% in women. Notably, colorectal cancer represents the most sharply escalating malignancy in Korea [2].

Hereditary syndromes are the source of approximately 5–15% of overall colorectal cancer cases. Hereditary colorectal cancer is divided into two types: (1) hereditary nonpolyposis colorectal cancer (HNPCC) and (2) cancers associated with hereditary colorectal polyposis, including familial adenomatous polyposis (FAP), Peutz–Jeghers syndrome, juvenile polyposis, and the recently reported *hMutYH* (*MYH*)-associated polyposis (MAP).

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A well-organized registry plays a central role in the surveillance and management of families affected by hereditary colorectal cancer syndromes [3–5]. Here, we discuss each type of hereditary colorectal cancer in Korea, with particular focus on the history of establishment, present status, activities, association with Cancer Gene Clinic in the hospital, and study results of the Korean Hereditary Tumor Registry. We additionally describe the Korean Health Insurance System, insurance support in gene testing, and the National Cancer Screening Program in Korea.

31.1 The Korean Polyposis Registry

In view of the need for official records for the effective management of FAP families, we organized the Korean Polyposis Registry in the Cancer Research Institute of Seoul National University in July 1990. The mission of the Korean Polyposis Registry was to identify individuals with FAP and those at risk of inheriting the disease, and provide optimal management and treatment plans. In collaboration with 26 major general hospitals throughout the country, we collected nationwide data on FAP patients. A modified version of the registration form from the Leeds Castle Registry was employed to obtain clinical data from a total of 72 families in the Korean Polyposis Registry [6].

31.2 Korean Hereditary Colorectal Cancer Registry

In the first meeting of the International Collaborative Group on Hereditary Non-polyposis Colorectal Cancer held in Amsterdam in 1990, initial criteria for the clinical diagnosis of HNPCC were established, based on family history [7]. In June 1991, we established the Korean Hereditary Colorectal Cancer Registry to record Korean families associated with HNPCC [8].

31.3 Korean Hereditary Tumor Registry

As the activities of the Korean Polyposis Registry and Korean Hereditary Colorectal Cancer Registry were expanded, we established the Korean Hereditary Tumor Registry in the Cancer Research Institute of Seoul National University College of Medicine in August 1993. The Korean Hereditary Tumor Registry includes a collection of clinical data on various types of hereditary tumors with collaborators from different departments in the hospital. We have screened the genes responsible for various hereditary tumor types, including colorectal, breast [9, 10], ovarian [9, 11], stomach [12–15], eye [16, 17], brain [18], bone [19], endocrine (multiple endocrine neoplasia syndrome) [20], and renal tumors [21].

31.4 Cancer Gene Clinic

The activities of the Korean Hereditary Tumor Registry include registration of new patients with hereditary tumors, mutation screening of the genes responsible for specific hereditary tumors, and surveillance of at-risk relatives by presymptomatic genetic diagnosis. These activities are performed in close cooperation with the Cancer Gene Clinic founded in Seoul National University Hospital (February 1997) and several hospitals that refer patients to the Registry. The Cancer Gene Clinic provides patients with disease information, genetic counseling, and a detailed management plan, such as the schedule of colonoscopy examination, time of operation, and treatment plans for other family members. These activities of the Cancer Gene Clinic are based on results of genetic screening from the Korean Hereditary Tumor Registry.

31.5 The Hereditary Tumor and Genome Research Workshop

Since 1995, the Korean Hereditary Tumor Registry has held an annual Hereditary Tumor and Genome Research Workshop to introduce updates of hereditary tumors and related research methods. To date, we have held 12 workshops involving presentations on 196 topics on hereditary tumor research, attended by 2,094 researchers.

31.6 Familial Adenomatous Polyposis

In 1990, we reported the results of a nationwide FAP surveillance of the Korean Polyposis Registry [6], in which 74 FAP patients were analyzed from 72 families, including three with Gardener's syndrome, 18 with Peutz–Jeghers syndrome, and 13 with juvenile polyposis. The mean age of patients at FAP diagnosis was 38 years, and colorectal cancer was detected in 58% of FAP patients. These results were consistent with the natural course of untreated FAP patients, and are typical in a region without a central polyposis registry.

In the past, we screened family members at higher risk of FAP, using procedures such as risk linkage analysis [22], polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), and protein truncation test [23]. Subsequently, we adopted denaturing high performance liquid chromatography (DHPLC) analysis as the primary screening protocol, followed by DNA sequencing in cases showing abnormal findings [24]. Currently, direct DNA sequencing is employed as the primary *APC* screening method.

The Korean Hereditary Tumor Registry contains information on a total of 623 individuals from 221 different families affected with FAP (98 families from Seoul National University Hospital and 123 from other general hospitals). In 2005, we reported germline mutations of the *APC* gene and genotype–phenotype correlations

in 83 unrelated Korean FAP patients [24]. Germline *APC* substitutions were identified in 59 cases (71%), including 34 frameshift, 19 nonsense, and six splice site mutations. All *APC* germline substitutions identified in this study involved truncations. Forty-seven (57%) of the 83 FAP patients had colorectal cancer at diagnosis, including 35 of 59 patients with *APC* gene mutations (APC-positive group) and 12 of 24 patients with no identified *APC* mutations (APC-negative group). The mean age at cancer diagnosis in the APC-positive and APC-negative groups was comparable (36.7 vs. 43.1 years), along with the mean number of polyps (1,085 vs. 833 polyps). Extracolonic manifestations were identified in 40 of the 83 (48%) screened patients. Four patients underwent operations for papillary thyroid carcinomas, and one individual was subjected to hepatic resection due to hepatoblastoma.

Recently, we adopted MLPA (multiplex ligation-dependent probe amplification) to detect large genomic deletions, and SNuPE (single nucleotide primer extension) to identify allelic expression loss in the *APC* gene. These methods are used to screen Korean FAP patients with no *APC* mutations identified from DNA sequencing.

31.7 Hereditary Nonpolyposis Colorectal Cancer

To date, the Korean Hereditary Tumor Registry has obtained information on 448 individuals from 78 different families affected with HNPCC (55 families from Seoul National University Hospital and 23 from other general hospitals), and 111 families with suspected HNPCC (104 families from Seoul National University Hospital and seven from other general hospitals).

We have screened 44 HNPCC and 97 suspected HNPCC Korean families for germline mutations in three MMR genes, specifically, *MLH1*, *MSH2*, and MSH6 [25]. Several previous studies have identified the MMR gene mutation in Korean HNPCC patients [26–30]. The HNPCC reports collectively indicate the presence of germline mutations in *MLH1*, *MSH2*, or *MSH6* genes of 22 (41.5%) of the 53 HNPCC families and 22 (19.8%) of the 111 suspected HNPCC families [25]. In Korean HNPCC patients, mutations in the *MLH1* gene were the most frequent, accounting for 91% of the MMR mutations identified in HNPCC and 50% in suspected HNPCC.

31.8 Founder Mutations in Korean HNPCC

Interestingly, the most frequent alteration in Korean HNPCC patients, c.1757_1758insC in *MLH1*, was a founder mutation inherited from a common Korean ancestor [25]. Eleven (35%) of the 31 families displaying *MLH1* substitutions harbored this mutation. Historical analysis revealed that all 11 families containing this mutation originate from the southern part of the Korean peninsula. Thus, screening for the *MLH1* c.1757_1758insC mutation, detected in a high proportion of Korean HNPCC families, should facilitate clinical counseling.

31.9 Suspected HNPCC

We have classified all registered HNPCC families into two subgroups, specifically, HNPCC and suspected HNPCC, using the Amsterdam Criteria II and revised criteria of suspected HNPCC. The Korean Hereditary Tumor Registry has proposed criteria for suspected HNPCC, and confirmed their validity in an international collaborative study [31, 32]. The original suspected HNPCC criteria I and II included families that did not fulfill the Amsterdam criteria, but with high risk of HNPCC. These criteria have recently been altered, since some suspected HNPCC families fulfilled the revised Amsterdam criteria, including some extracolonic cancers. The revised standards for suspected HNPCC are as follows:

- At least two HNPCC-associated cancers in first-degree relatives (colorectum, endometrium, small bowel, urinary tract), and
- Multiple colorectal tumors or
- At least one HNPCC-associated cancer diagnosed before 50 years of age or
- Development of an accompanying cancer in family members (stomach, biliary, pancreas, ovary)

We conducted an international collaborative study showing that the value of the revised suspected HNPCC criteria is equivalent to that of old suspected HNPCC criteria in the selection of candidates for genetic testing [32]. Data on the mutational status of 393 HNPCC suspected families were collected from ten different institutes. Two hundred families were categorized into old suspected HNPCC criteria (142 into criteria I and 58 into criteria II) and 193 families into Amsterdam criteria I. Out of the 142 families, 24 fulfilled Amsterdam criteria II, as the data were reclassified according to revised standards, leading to an increase in the proportion of families fulfilling the Amsterdam criteria by 12.4%.

We analyzed the clinical characteristics of 93 patients from 39 Korean suspected HNPCC families. The clinical characteristics of suspected HNPCC are generally similar to those of HNPCC or between non-hereditary colorectal cancer and HNPCC in terms of mean age of diagnosis, right-sided predominance in tumor location, and the existence of synchronous/metachronous tumors [33].

31.10 Gastric Cancer in FAP and HNPCC

Interestingly, three out of 72 Korean FAP patients had gastric cancer at diagnosis and the standardized incidence ratio (calculated as the number of observed cases divided by the number of expected cases) was 6.9. FAP patients were at a ~7-fold increased risk for gastric cancer, compared to the general population in Korea [34].

Korean HNPCC patients also display a high incidence of gastric cancer [35]. Korea is one of the most prevalent areas in the world for gastric cancer occurrence. Therefore, it is natural to expect a different, possibly increased risk of gastric cancer in Korean HNPCC patients, compared to that derived from a gastric cancer

nonendemic area. Consistent with this theory, Korean FAP patients were at ~7-fold increased risk for gastric cancer, compared to the general population [34]. Similarly, the risk of gastric cancer in Korean HNPCC patients was 2.1-fold greater than that in the general population. Additionally, the relative risk of gastric cancer in the younger generations was significantly greater, specifically, 11.3- and 5.5-fold for individuals in their 30s and 40s, respectively.

31.11 Peutz–Jeghers Syndrome/Juvenile Polyposis

At present, the Korean Hereditary Tumor Registry contains information on 30 Korean patients (21 families) affected with Peutz–Jeghers syndrome, and four unrelated patients with juvenile polyposis [36].

Out of the 30 patients registered in the Korean Hereditary Tumor Registry, we screened ten individuals with Peutz–Jeghers syndrome. Germline mutations of the *STK11* gene were identified in five (50%) Korean PJS patients, including three missense, one frameshift, and one aberrant splicing [37]. Out of four patients with juvenile polyposis, we observed three different germline mutations of *SMAD4* (*DPC4*) in three patients, and a novel *BMPR1A* germline mutation in the other patient with no *SMAD4* mutations [38, 39].

31.12 *MYH*-Associated Polyposis

Since the initial report on a British Caucasian family with multiple colorectal adenomas and carcinoma displaying heterozygous *MYH* biallelic mutations [40], a number of investigations on MAP have been conducted. However, limited data are currently available on MAP in Asia. Biallelic germline mutations of the *MYH* gene were identified in FAP patients containing no discernable mutations in the *APC* gene and those with 15–99 adenomas, at a frequency of 7–42%. Most previous studies on European Caucasian patients revealed autosomal recessive inheritance of MAP. Two hot-spot mutations, p.Y165C and p.G382D, were the most frequent germline alterations of *MYH* [41–43]. Insufficient information is available on the genotypic and phenotypic characteristics of MAP in Asian patients at present.

We screened for *MYH* mutations in 14 patients with 15–99 adenomatous polyps in the colorectum. The frequency of biallelic *MYH* mutations was 14.3% (2/14) [44]. A 39-year-old male patient with biallelic *MYH* mutations (p.G272E and p.A359V) received total proctocolectomy for rectal cancer and 36 colorectal polyps. A 58-year-old female patient with biallelic *MYH* mutations (p.Q253X and p.Q440P) received right hemicolectomy for ascending colon cancer and 16 colonic polyps. No biallelic *MYH* mutations were detected in 32 patients with 10–14 colorectal polyps, 16 FAP patients with no *APC* germline mutations or 96 normal

controls. Apart from p.IVS10-2A>G., five out of the six *MYH* variants detected in our experiments have not been reported previously, according to the *MUTYH* database in HGMD (The Human Gene Mutation database; http://archive.uwcm.ac.uk/uwcm/mg/hgmd/search.html). Interestingly, we did not identify any Y165C or G382D hot-spot mutations, reported most frequently in previous studies. These results support ethnic and geographic differences in the mutational spectrum of the *MYH* gene.

31.13 Korean Health Insurance System

The Korean health insurance system was institutionalized in July 1977. The insurance system was expanded gradually, until July 1989, when the whole population was covered by health insurance. In a population of about 48 million in Korea, approximately 3% with very low income are covered by the Medical Aid Program financed by government budget.

When an insurance beneficiary is taken ill or injured, and demonstrates symptoms to warrant medical care, institutions directly provide medical services for the diagnosis and treatment of the illness or injury, or prevention of the illness in question. After providing medical care, the institution submits a claim to the Health Insurance Review Agency (HIRA) for the review of medical fees. Subsequently, the National Health Insurance Corporation (NHIC) reimburses the claim, based on the review results from HIRA.

31.14 Insurance Support in Gene Testing

Among the genes responsible for human hereditary cancers, the only item covered by the national health insurance system is mutational screening of *BRCA1* and *BRCA2* genes in breast cancer patients in Korea. At present, NHIC coverage is not extended to mutation screening for at-risk relatives of the identified mutation carriers of *BRCA* genes. Mutation screening of the *APC* gene in FAP and MMR genes in HNPCC has been approved, but is not yet covered by the insurance system.

31.15 National Cancer Screening Program

In Korea, the National Cancer Screening Program has been in operation since 1999, with a view to improve early detection of cancer and reduce patient mortality. Every individual belonging to the lower 50 percentile brackets of income can be screened for early detection of cancer in Korea. In 2002, this program included screening for breast, cervix, and lung cancer, and gastric cancer was included in 2003. Screening

for colorectal cancer was initiated as part of the National Cancer Screening Program in 2004.

Guidelines for colorectal cancer in the National Cancer Screening Program target all adults over 50 years of age, and recommend fecal occult blood test (FOBT) every year, followed by colonoscopy or barium enema in case of abnormal FOBT results. We expect that the colorectal cancer screening program by the Korean government should contribute, to some extent, to the detection of newly developing hereditary colorectal tumors.

31.16 Summary

Since the establishment of the Korean Polyposis Registry, we have concentrated on obtaining detailed family history, accurate genetic diagnosis, and specialized approaches for patients affected with hereditary colorectal tumors in clinical practice. Our data collection has been significantly assisted by recent developments in medicine and molecular biology.

In this report, we discuss the history of the Korean Hereditary Tumor Registry, and summarize the clinical and genetic characteristics of hereditary colorectal tumors in Koreans, based on previous studies over the past two decades. The genotype and phenotypes of FAP are consistent with data from earlier reports from other areas worldwide. However, higher risk for gastric cancer is a distinctive characteristic of Korean FAP and HNPCC patients. The *MLH1* gene with the founder mutation, c.1757_1758insC, is predominantly responsible for HNPCC development in Korea. In MAP, no Y165C or G382D hot-spot mutations have been identified out of the six variants of *MYH* in our study, which are reported most frequently in Caucasians. Our results support ethnic and geographic differences in the mutational spectrum of the *MYH* gene.

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Chapter 32
Across Culture and Health Systems: America

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Keywords Across • Culture • Health • Systems • America

32.1 Introduction

Recognition of families with high penetrance of young-onset colorectal cancer has existed for over a century [1–3]. However, concerted efforts to define these high-penetrance diseases occurred much later [4, 5]. Once the molecular basis of hereditary colorectal cancer syndromes was elucidated, the clinical and research efforts toward identification, clinical care, and management of individuals at hereditary risk for colorectal cancer experienced an exponential growth within the last two decades.

Studies of hereditary colorectal cancer syndromes such as familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, and the hamartomatous polyposis syndromes have demonstrated that these syndromes occur in similar incidence throughout the world. Genetic testing for these syndromes has become widely available in the USA within the last 15 years. This chapter provides a summary of the provision of familial cancer risk assessment services and availability of cancer genetic testing in the USA.

In order to understand the context in which clinical cancer genetic counseling and testing is available, we will first review the unique aspects of the American health care system. Identification of high-risk individuals and families were initially coordinated by research studies; however but with public awareness for the genetic basis of disease, health care providers (HCPs) have the responsibility to identify and evaluate at-risk individuals. A widening body of professional organizations and national committees publish criteria on the identification and recommendations for management of individuals with a hereditary predisposition to colorectal cancer.

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Provision of cancer genetic services is mostly through institutionally supported clinical cancer genetic programs. Lastly, we will discuss concerns regarding genetic discrimination within the framework of the US health care system.

32.2 Overview of the American Health Care System

In order to appreciate the delivery, uptake and clinical aspects of cancer genetic counseling in the USA, it is important to understand the American health care system. One of the most unique aspects of health care in the USA may be the private-payer model for coverage of medical services. This is in contrast to the predominance of the public sector payment model adopted by Canada, Australia, New Zealand and most European countries.

The majority of Americans receive some form of health insurance. Private health insurance encompasses both employer-sponsored insurance and individuals who self-purchase a plan. According to the US Census Bureau, employer-sponsored health care insurance covers approximately 60% of the US population, while 9% purchase insurance individually [6]. Less than one-third of Americans are insured by one of two federally funded health insurance plans Medicare and Medicaid for aged/disabled people and low income earners, respectively. Unfortunately, nearly one-sixth, or 45.7 million people, of the American population is uninsured [6].

Private health insurance companies provide coverage for nearly three-fourths of insured Americans. Preferred Provider Organization (PPO) insurance plans are the most common form of private-payer in the US. Nearly 60% of individuals with private health plans utilize PPO plans [7]. PPO plans comprise a network of physicians and HCPs authorized by an insurance company to which the individual pays premiums. Patients can choose to see HCPs of any specialty as long as they are within the network. The network of contracted HCPs agrees to provide services at discounted rates to members of PPO plans. However, if a procedure, medication or appliance costs the insurance company more than what they agreed to pay, the patient is often responsible for any additional cost. In some cases, particularly when the patient has significant illness, the patient may be left with a significant financial burden [7].

Health Maintenance Organization (HMO) plans provide for 21% of those with private health insurance plans [7]. Patients purchase a contract with an HMO plan with payment of a small premium. Most basic medical care is covered under this premium and there are often no, or very small, co-payments. All patients covered by HMOs are required to have a "gateway" practitioner called a primary care physician (PCP). This practitioner is responsible for seeing the patient and providing any necessary referrals. Patients may not seek care by a specialist and have that care be considered for coverage unless the PCP determines it is necessary. The patient may also have to either take the exact referral to the specific physician from the PCP's referral list, or may be required to find a suitable practitioner within the HMO system. If a particular specialty is not covered by a participating practitioner, the HMO may not provide any payment for treatment. If "out-of-network"

practitioners must be used for any portion of care, the patient will be responsible for the cost in its entirety. HMOs tightly integrate the delivery of health care and payment of services [7].

Medicare and Medicaid are two of the largest government-funded health care systems in the USA. Both systems are actually managed by private insurance companies, but the funding is established and criteria for coverage and payment are regulated by the US Department of Health and Human Services. Medicare is an entitlement program designed to provide medical support to individuals who are above age 65 (age of retirement) and individuals who are permanently disabled. Individuals participating in Medicare must also pay premiums, although they are significantly reduced by the infusion of funding through Medicare Insurance paid as part of the Income Tax system by those individuals who are currently working. The Medicare Program provides Medicare Part A which covers inpatient hospital bills, Medicare Part B which covers doctor's services, outpatient care and some preventative services, and Medicare Part D which covers prescription drugs.

Medicaid is a needs-based program designed for individuals who are financially unable to pay for their health care and families who fit into designated eligibility categories (age, pregnancy, disability, blindness, and poverty). Medicaid funding is jointly provided by the federal and state governments, but regulation is provided at the state level, thus coverage may vary from state to state. Medicaid covers a wider range of health care services than Medicare. Criteria for enrollment in Medicaid are relatively strict, and in some cases, the child or children in a given family may qualify but the parents will be left uninsured [8].

Despite the availability of employer-sponsored health insurance plans and public-payer programs, 15% of Americans are uninsured [6]. The majority of the uninsured (85%) are native or naturalized citizens. Over eight in ten uninsured come from working families and almost 70% are from families with one or more full-time workers. Even though employers offer insurance plans, many workers cannot afford the high premiums. Adults are more likely to be uninsured than children since low-income children often qualify for Medicaid coverage [9].

Coverage for cancer risk assessment services and genetic testing vary significantly due to the different payment programs for health care services. In clinical practice, the majority of patients insured by private health care plans receive varying degrees of coverage for genetic services. This is in part because of the lack of standard criteria for coverage and level of payment for services. Criteria for coverage of Medicare and Medicaid services are established by the Centers for Medicare and Medicaid Services. Reimbursement of physicians, clinical laboratory services and related health care services and supplies is based on a fee-for-service basis and is posted online as a comprehensive listing [10]. Coverage for genetic services by these federal programs is based on stringent criteria.

Cancer genetic testing has only become widely available in the USA for approximately 15 years. Challenges remain regarding coverage of genetic testing and genetic counseling services by insurance companies. In addition, genetic counseling is primarily regarded as a preventative service, in that screening is performed for otherwise healthy individuals who have a genetically based predisposition for

certain illnesses, diseases, and even cancer. Because genetic counseling is considered by insurance companies to not be a treatment-based modality, and due to the high costs of genetic testing, many individuals are concerned that coverage for these services would not be provided. This in turn adds one more barrier for at-risk individuals to pursue appropriate risk assessment and genetic testing.

32.3 Identification of High-Risk Individuals

Identification of high-risk families for colorectal cancer was initially through recruitment to research studies, many of which evolved into cancer registries. Research studies enabled high-risk families to have access to knowledgeable scientists and clinicians who were familiar with the unique aspects of the cancer syndrome. Today, individuals with inherited predisposition to colorectal cancer are commonly identified outside the research setting. This is largely due to increase of public awareness and knowledge that disease risk can be influenced by genetic risk factors. Initiation of the Human Genome Project in 1991 drew mainstream attention to the potential impact of genetic risk on diseases. Numerous professional organizations have responded to the concern for appropriate use of genetic information for identification and management of individuals with inherited susceptibility to cancer by establishing recommendations. These include the American Society for Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN), and various professional organizations such as the National Society of Genetic Counselors (NSGC), American Gastroenterological Association, and Society of Gynecologic Oncologists [11–17].

Individuals concerned about their family history of cancer may obtain access to genetic services in several ways. Among the National Cancer Institute (NCI)-designated cancer centers, the vast majority of referrals (96%) for familial cancer risk assessment come from within the given institution [18]. ASCO advocates the role of the oncologist in identifying patients affected by cancer due to an inherited predisposition [19]. As part of the initial medical history intake, oncologists should obtain cancer family history information, and if suspicion is raised for a hereditary cancer syndrome, appropriate referrals for evaluation should be taken. Gastroenterologists and surgeons also share responsibility in identifying at-risk patients for familial cancer risk assessment [20, 21]. Some centers have established criteria, based on the patient's personal or reported family history, to facilitate identification of patients appropriate for familial cancer risk assessment.

Within the community clinical setting, awareness of cancer risk assessment and referrals to genetic services are much lower. A study within the community setting found that 59% of physicians were aware of the availability of local cancer genetics program [22]. Furthermore, specialty physicians were more aware of the genetic services than PCPs. With a gastroenterology clinic, approximately one in four patients were found to be eligible for more comprehensive evaluation of their personal medical and family history for inherited susceptibility for colorectal cancer [23]. The majority

of these physicians inquired about family history, but the recognition of at-risk individuals and subsequent referral to genetic counseling was low [23, 24]. In general, knowledge about hereditary colorectal cancer syndromes and availability of genetic testing was sub-optimal among a survey of gastroenterologists and PCPs [26, 27]. Significant efforts continue to be made to assist HCPs in the community setting to identify at-risk individuals for appropriate cancer genetic risk assessment [28–30].

Patients may also initiate self-referral for risk assessment, with 89% of cancer centers accepting patients without referral by a health care provider [18]. These individuals may identify cancer genetic health professionals from the internet. These include the National Society of Genetic Counselors (www.nsgc.org), National Cancer Institute (www.cancer.gov/search/geneticsservices/) and GeneTests (www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests).

Individuals who request self-referral for genetic services may be limited in accessing such services depending on their insurance coverage. Those covered by HMOs must be referred by their PCPs since genetic services are considered a specialty service. If the PCPs approve the referral, they must place a formal request to the HMO for the individual to have access to the genetics provider. There may a wait time of a few days to a few weeks before receipt of approval by the HMO. For individuals insured by PPOs, genetic risk assessment would be covered as long as the genetic providers are within the PPO network. It is often the insured's responsibility to determine whether the intended genetics provider is within their network. Finally, individuals covered by the federally funded Medicare and Medicaid programs receive coverage for genetic services if there are established criteria for such services.

32.4 Provision of Clinical Cancer Genetic Services

Comprehensive cancer clinics, either dedicated to hereditary colorectal cancer syndromes or general clinical cancer genetics, have been established to serve the needs of high-risk individuals and their families. A 2004 survey of NCI-designated cancer centers revealed that over 80% provided familial cancer risk assessment services [18]. Currently 20 of the 21-member institutions within the NCCN provide cancer risk assessment services [13].

The risk assessment of an individual or family for a hereditary cancer predisposition syndrome can be provided by a variety of health care professionals. Genetic counselors are medical professionals trained to communicate and help individuals adapt to the medical, psychological and familial implications of genetic conditions or diseases [31]. There are currently 2,100 genetic professionals registered as full members of the National Society of Genetic Counselors [32]. Forty percent of genetic counselors reported to specialize in cancer genetics. Genetic counselors work in a variety of clinical settings, with the majority working in university medical centers or hospital/medical facilities. Genetic counselors often work within a clinical team, under the supervision of a medical director. The background and clinical expertise of the medical director varies between centers, and may include

medical geneticists, oncologists, or surgeons. Patients may be seen by either a genetic counselor or medical physician, or both. Over half (54%) of the genetic counselors reported that a physician personally sees their patients [32]. The majority of the coordinated visits occur with a medical geneticist (53%), while oncologists comprise 15%.

Several excellent reviews outline the genetic counseling process for familial cancer risk assessment in the USA [14, 33]. Significant time should be dedicated to the initial genetic consultation visit to elicit and review the medical and family history, complete the risk assessment, and if cancer susceptibility is suspected, provide anticipatory guidance for genetic test results and interpretation [12, 14]. In 2003, ASCO published recommendations for genetic testing for cancer susceptibility, placing emphasis for the clinician to discuss all the elements of the informed consent process during the pre-test counseling session [12]. Whether testing is offered or pursued, a personalized plan for cancer screening is recommended based on the reported family cancer history and personal risk factors. When genetic testing is pursued, the discussion would be deferred until disclosure of the results and final risk assessment.

In many large academic institutions, hereditary colorectal cancer clinics have been established with a core team of multidisciplinary specialty clinicians available to see high-risk individuals for risk assessment and management [34–36]. Ideally, staffing should include a gastroenterologist, colorectal surgeon, and genetic counselor or geneticist. Services provided by a wound ostomy nurse, psychologist and social worker may provide additional benefit to the patient and families. Overall, patients seen in a comprehensive cancer genetics clinic reported satisfaction with the services (90%) and would recommend the services to friends and family [37].

An innovative model recently emerged for individuals to access genetic counseling services from any location using state-of-the-art telephone and internet technology. Informed Medical Decisions, Inc. is the first national network of independent genetic counselors available to provide family cancer risk assessment and make recommendations for appropriate genetic testing [38]. Individuals schedule a genetic consultation appointment either through the internet website or placing a telephone call. The genetic counseling can be scheduled at a time convenient for them. Personal, medical, and family history is obtained through a secured online web interface. The information is reviewed over the telephone by a board-certified genetic counselor at the scheduled time. Individuals must provide contact information to a physician so that all consultation summary notes and recommendations are communicated to their local health care provider. If genetic testing is pursued, the genetic counselor works with the individual's doctor for coordination of testing. Follow-up services, including post-test counseling and interpretation of results, are also provided. This delivery model for genetic services can be appealing for individuals who live in areas that have limited availability to cancer genetic specialists or individuals who have limited mobility or desire to undergo genetic counseling in the comforts of their home and at convenient times.

Research registries for hereditary cancer syndromes, or the more specific hereditary colorectal cancer syndromes, remain associated with the cancer genetic clinical services. Some clinical programs expanded from the research efforts, while many registries were established after initiation of the clinical genetic services.

In 2004, a survey of cancer genetic registries in the USA revealed 30 registries, the oldest of which exist at Johns Hopkins University in Baltimore, Maryland, Creighton University in Creighton, Nebraska, the Cleveland Clinic in Cleveland, Ohio, and the University of Texas M. D. Anderson Cancer Center in Houston, Texas [39]. These established registries ranged from 14 to 29 years, whereas the median age of registries was 5.5 years. Most of the registries existed within cancer centers affiliated with the NCCN. A current listing of active hereditary colorectal cancer registries is available through the Collaborative Group of the Americas on Inherited Colorectal Cancer (www.cgaicc.com).

Billing practices for genetic counseling services vary between the facilities. Patients are billed for the services and may choose to self-pay or use their health insurance carrier. The variable amount billed for the genetic consultation may be based upon the medical facility, amount of time spent with the patient, complexity of the risk assessment, and whether the individual was seen by a physician or genetic counselor. The level of coverage provided by the insurance companies varies, with most insurances making payment for the genetic consultation visit similar to any other medical office visit. Though rare, some insurance policies have exclusions for genetic counseling services. In these cases, patients are required to make direct payment if they wish to proceed with the clinical services. Some patients decline to pursue genetic risk assessment due to prohibitive financial costs.

Many of the major health insurance companies are becoming aware that genetic screening and preventative services actually reduce their costs in the long run. Though it is a slow process, private and public insurances are beginning to embrace genetic screening, counseling, and medical interventions for management of many hereditary cancer syndromes. UnitedHealthcare (UHC), one of the largest health insurance companies in the US, recently adopted a new policy, effective August 19, 2009, which ensures that all insured individuals have access to board-certified genetic counselors. The genetic counselors would work with the ordering physician and patient to provide guidance to ensure the appropriate individuals are offered genetic testing and furthermore, assist with interpretation of the results and treatment options [40]. UHC is contracted with Informed Medical Decisions for genetic counseling services when genetic testing is recommended or ordered by the individual's local healthcare provider.

If genetic testing is recommended for the appropriate individual and is pursued, patients incur the additional cost for the genetic testing. Coverage for genetic testing also significantly varies between insurance plans. While Medicare is a federal program, Medicare does not have national coverage determination for genetic testing for Lynch syndrome or FAP. However, local coverage determinations for coverage of genetic testing for these syndromes exist, and may be found online [10].

Criteria have been established in some local coverage determinations for genetic testing for Lynch syndrome, FAP, and MYH-associated polyposis. Individuals must meet the stringent criteria in order for genetic testing to be a covered benefit, after which it would be covered at 100%. Currently, the Medicare criteria for Lynch syndrome genetic testing qualifies that, individuals must have a history of colorectal or endometrial cancer AND have a family history which meets with Amsterdam criteria. Unfortunately, some individuals with a significant family history, which is highly suggestive for Lynch syndrome, may not meet the criteria (such as an individual with personal history of transitional cell carcinoma of the ureter and Amsterdam-positive family). Since most individuals covered by Medicare are retired and have limited income, the cost (ranging from $1,000 to $3,000) could be prohibitive for them to undergo genetic testing. Many private health insurance providers also set forth criteria for coverage of genetic testing. If the individual does not meet the criteria, a request for medical review by the designated physician or health care may be made to appeal for the individual to be approved for genetic testing. This process can often take several weeks before final determination for coverage of genetic testing is made. Coverage for genetic testing by private health insurance plans are also discrepant across plans. In the author's clinical experience, most insurance plans cover some cost of the genetic testing.

Individuals who have no health insurance coverage for healthcare have limited access or even awareness of cancer genetic services and testing. Due to the high cost of genetic counseling and genetic testing, most individuals without health insurance cannot afford these services. Forty four percent of eligible patients were not referred for cancer risk assessment due to physician concerns for lack of insurance coverage [22]. Patients must therefore weigh the cost of their personal financial responsibility for the test with the benefit of having the genetic test result incorporated into their extended medical care.

An alternative avenue for individuals who do not have medical health insurance, or are unable to afford their co-insurance, for genetic services is through participation in research studies. Research registries or studies often have funding available for coverage of genetic counseling and/or genetic testing. These opportunities have become more limited with the growing availability and coverage for genetic services by health insurance plans. Still, some cancer centers maintain sufficient grant-funding for provision of genetic services, allowing eligible study participants to receive risk assessment services, genetic testing, and even access to colonoscopies or other medical procedures under the research protocol.

In summary, widespread efforts have been made to educate HCPs to identify at-risk individuals for hereditary colorectal susceptibility syndromes. Genetic counseling services are available through many different clinical settings, ranging from multidisciplinary risk assessment and management clinics to internet and telephone-based genetic counseling. Hereditary colorectal cancer registries remain an important facet of comprehensive cancer centers. Access to and coverage for these services varies significantly for Americans, in part due to the health care system. Individuals who may benefit from genetic counseling and testing often have to incorporate the cost of the financial burden of the service into their decision-making process.

32.5 Genetic Discrimination

The concern for genetic discrimination may be a unique artifact of the American health care system. Access to health insurance for coverage of basic health care is available to most Americans. However, not all of these individuals choose to undergo genetic testing, even with adequate insurance coverage of testing. Another aspect of the decision-making process for genetic testing is the concern for genetic discrimination.

Several studies have documented the widespread fear of genetic discrimination among health care professionals [41, 42]. One impact of HCPs' fears is reduced referral for cancer genetic risk assessment [22, 48]. Brandt et al. found that 31% of patients were not referred for cancer genetic services due to physician fears of genetic discrimination [22].

A 1996 survey of members of genetic support groups found that 22% believed they were refused health insurance as a result of a genetic disorder [43]. A more recent survey of unaffected mutation-positive carriers for a hereditary cancer susceptibility syndrome found that 2 of 47 individuals experienced denial of coverage by private health insurers. These fears have been corroborated by several studies of insurers [49], which found that health insurers would deny coverage, charge higher premiums, or place certain restrictions on the policy [44, 45]. Fear of genetic discrimination has also prevented individuals from undergoing genetic testing for inherited cancer susceptibility. Despite the benefit of knowing one's risk status for inheritance of the familial mutation for hereditary cancer and the ability to undergo high-risk cancer surveillance if needed, individuals report that they or their family members did not undergo genetic testing because of discrimination [49, 50].

In response to consumer and HCPs' concerns, states began to enact laws against genetic discrimination. In 1991, Wisconsin became the first state to enact legislation prohibiting health insurers from requesting genetic information or using such information to determine eligibility or risk classification. Currently all but three of the 50 states have passed laws pertaining to genetic discrimination; however, each state varies in whether the protections cover requirement of genetic test/information, eligibility and risk classification by insurers [25]. More than 30 states have laws which prohibit employers from practicing genetic discrimination. Since these are state laws, the coverage is not comprehensive for individuals who move from one state to another. They may face a new set of laws and this may be particularly distressful to individuals in today's mobile society.

One of the first federal legislative attempts to address concerns regarding genetic discrimination was the enactment of the Health Insurance Portability and Accountability Act (HIPAA) of 1996. HIPAA provided limited protection from genetic discrimination in group health insurance and employment. Under HIPAA, genetic information was regarded as part of an individual's protected health information and that genetic information cannot be considered a pre-existing condition in the absence of a current diagnosis [46]. HIPAA was not comprehensive in its protections, however. Rather than charging higher premiums for the individual, there were no protections in HIPAA preventing insurers from charging the entire

group more. Insurers were not prohibited from requiring individuals to undergo genetic testing or collecting genetic information.

In an effort to fill in the gaps from the patchwork of previous federal and state legislations, the federal Genetic Information Nondiscrimination Act (GINA) of 2008 was signed into law in May 2008 [47]. Moreover, GINA was not passed without struggle as it took more than 12 years of deliberation by the US Congress. GINA prohibits health insurers and employers from asking or requiring an individual to undergo genetic testing and from using genetic information in establishing insurance rates or making employment decisions. GINA does not prohibit establishment of insurance eligibility and rates based on current health status, apply to life or disability insurance, or to members of the military. GINA defines genetic information as genetic test results of an individual or their family member (up to and including fourth-degree relatives), any manifestation of a disease in a family member, and participation of an individual or their family member in a program which includes genetic testing, counseling or education [47]. Under the protection of GINA, individuals could undergo predictive testing for hereditary cancer syndrome, and other genetic tests such as somatic tumor testing to guide treatment-based recommendations or carrier screening for autosomal recessive conditions [48].

GINA has been championed as the first major civil rights legislation of the twenty-first century [48]. The hope is that GINA will provide reassurance to individuals who were previously hesitant or concerned to pursue genetic testing or participate in genetic research studies.

GINA received considerable publicity when it was signed into law; however, genetic professionals need to continue to educate their colleagues in health care and patients about laws protecting patients from genetic discrimination.

32.6 Summary

This chapter summarizes the access, delivery, and update of genetic services for individuals with hereditary colorectal cancer syndromes. These individuals and their at-risk family members should ideally be managed within high-risk multidisciplinary colorectal cancer genetic programs. Access to these services varies considerably, in part due to physical location and accessibility but also to provision of coverage by health insurers. With passage of the Genetic Information Nondiscrimination Act of 2008, hopefully there will be one less barrier for at-risk individuals to pursue appropriate risk assessment and genetic testing.

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Part X
Psychosocial Aspects of Hereditary Colorectal Cancer

Chapter 33
Psychological Impact of Genetic Counseling and Testing for Hereditary Colorectal Cancers

Susan K. Peterson

Abstract Lynch syndrome/Hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP) are two hereditary cancer syndromes that confer an increased risk for colorectal cancers. Lynch Syndrome and FAP together account for about 5% of all CRC. Deleterious germline mutations associated with these syndromes have been identified in mismatch repair (MMR) genes (i.e., *hMLH1, hMSH2, hMLH6, PMS1*) for Lynch syndrome and in the *APC* gene for FAP. Genetic testing enables health care providers to identify individuals who carry such mutations and thus have a risk for developing colorectal cancer and other tumors that substantially exceeds the general population risk for this disease. A primary benefit of genetic testing is the ability to offer targeted options for cancer risk management to persons at increased risk due to an inherited susceptibility.

Since genetic testing for Lynch syndrome and FAP became clinically available over a decade ago, psychosocial research has focused on understanding individuals' motivations and decisions regarding genetic testing, the psychological impact of genetic risk notification, effects on family and interpersonal relationships, and factors that influence the uptake of risk reduction options (e.g., screening, risk-reducing surgery, or chemoprevention). This chapter will review the literature on these topics for Lynch syndrome and FAP. Findings from psychosocial research on Lynch syndrome and FAP can guide clinicians in understanding why people seek genetic counseling and testing, what they hope to gain from it, and how they cope with the results of testing and integrate that information into cancer prevention and treatment decisions.

Keywords Hereditary non-polyposis colorectal cancer (HNPCC) • Lynch syndrome • Familial adenomatous polyposis (FAP) • Genetic testing • Genetic counseling • Psychosocial • Psychological • Quality of life • Family communication • Adherence • Decision-making

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33.1 Uptake of Genetic Counseling and Testing for Lynch Syndrome and FAP

Genetic testing for inherited cancer susceptibility is a multi-step process that involves several decision points, including whether to seek counseling, undergo mutation testing, and receive test results. After genetic test results are disclosed, additional decisions must be made about whether and when to share results with family members, health care providers or others, and about risk management options such as screening, risk-reducing surgery, or chemoprevention. Understanding individuals' motivations to undergo genetic testing, as well as reasons for declining testing, is critical to maximize the usefulness of genetic testing in clinical practice.

A growing number of studies have examined uptake rates for genetic counseling and testing for gene mutations associated with Lynch syndrome and FAP, and have identified demographic, clinical, and psychosocial factors associated with testing participation. It is important to note that comparing uptake rates across studies may be challenging because of methodological differences. Many studies recruited participants from familial cancer registries or clinical settings, such as cancer genetics or oncology clinics, and free genetic counseling and testing was frequently offered as part of research protocols. In addition, there are many points in the genetic counseling and testing process at which an individual may decline, and a standard methodology for defining and reporting uptake rates has not been established [1].

33.1.1 Genetic Testing for Lynch Syndrome-Associated Mutations

Genetic testing uptake rates for Lynch syndrome-associated mutations have varied widely, ranging from 14 to 59% [2–5]. Factors such as cost and the context in which counseling and testing were offered may have influenced participants' decisions to undergo genetic testing in these studies. For example, uptakes rates tend to be highest (i.e., 36–59%) among studies that offered free genetic counseling and testing as part of a research protocol [2, 4–7].

Factors associated with the decision to undergo genetic testing for Lynch syndrome susceptibility included having a personal history of cancer, a greater number of affected relatives, a greater perceived risk of developing colorectal cancer, and more frequent thoughts about colorectal cancer [2, 4, 5, 7]. While uptake rates appear similar among men and women, those who underwent testing were more likely to be employed and to have higher educational levels compared with decliners [4, 5, 7]. Participation in genetic counseling to learn about Lynch syndrome-associated cancer risk has been correlated with having greater perceived social

support [8], and the desire to learn about one's mutation status may be motivated by the belief that testing will help family members [8].

Less is known about the characteristics of persons who decline genetic testing for Lynch syndrome, as these individuals also may be less likely to respond to surveys regarding their testing decisions. Test decliners may be more likely to report depressive symptoms, non-adherence to colorectal cancer screening, and a lower perceived ability to cope with mutation-positive test results [4–7]. Other reasons cited for not seeking genetic counseling or testing have included concerns about potential insurance discrimination, worries about how genetic testing would affect one's family, and concerns about anticipated emotional reactions to genetic test results [2].

The recommended approach to genetic testing for Lynch syndrome optimally begins with microsatellite instability (MSI) testing in tumors of affected persons who meet the revised Bethesda guidelines for this syndrome, and it is expected that the clinical use of MSI testing to screen high risk colorectal cancer patients will increase [9, 10]. However, we know little about patients' awareness, understanding or preferences for MSI testing. One study found low levels of knowledge and awareness of MSI testing among a sample of patients who met the revised Bethesda criteria and who were offered MSI testing [11]. In general, patients reported positive attitudes about the benefits of MSI testing; however, those with higher levels of cancer-specific distress were perceived with a greater number of barriers to having MSI testing. These findings suggest that colorectal cancer patients may benefit from improved education about MSI testing in order to improve understanding and facilitate informed decision making about the test.

33.1.2 Genetic Testing for APC Mutations in FAP

Genetic testing for *APC* mutations that cause FAP is one of the few circumstances in which testing for hereditary cancer predisposition in children is recommended as standard of care. Genetic testing for adult-onset hereditary cancers is generally not advised for minors because of the lack of proven medical benefit of such testing, the potential psychosocial risks, and the desire to preserve minors' ability to exercise autonomy in decision making about testing once they reach the age of consent. However, *APC* gene testing is an important component of medical management for FAP. There is a clear and immediate benefit to testing children because testing can identify an inherited predisposition in those who are presymptomatic, signaling the need to initiate endoscopic screening at puberty (when polyps typically develop) as well as to consider planning for risk-reducing colectomy. Alternately, genetic testing can identify children of FAP-affected parents who are not *APC* mutation carriers and who do not need to worry about annual colonoscopies and future surgery, resulting in cost savings and possible avoidance of psychosocial harms. Important issues to consider when offering clinical *APC* testing are whether the child is of

sufficient age, maturity and psychological stability to understand the reasons for genetic testing, and the implications of the test result. [12]

Uptake rates for APC genetic testing may be higher compared with rates for MMR gene mutation testing in Lynch syndrome, ranging from 65 to 95% across studies [13–15]. Testing rates reported for minors were among the highest in these studies at 95–96% [14, 15]. In a cross-sectional study of adults at risk for or diagnosed with FAP, 24% of clinically unaffected adults had undergone genetic testing compared with 79% of clinically affected adults who had had testing, which may reflect the use of *APC* testing to confirm an FAP diagnosis in persons with clinical symptoms [13]. The decision to undergo testing may be motivated by concerns about one's own future health and/or the health of one's children [16], and one study reported that 42% preferred to have children tested at birth rather than in later childhood [13].

Concerns about the future health of children also may motivate individuals with FAP to consider prenatal testing to identify whether an embryo or fetus is affected as well as preimplantation genetic diagnosis (PGD) to prevent the transmission of *APC* mutations. Hypothetical interest in prenatal genetic testing ranged from 75 to 95% in surveys, and hypothetical interest in PGD ranged from 61 to 95% [13, 17]. While these studies indicate a fairly high level of potential interest in prenatal testing or PGD, the actual prevalence of these procedures among FAP-affected persons is unknown. Nonetheless, these findings suggest that reproductive decision making, including prenatal diagnostic options, may be relevant topics to explore during FAP genetic counseling.

In summary, the decision to undergo genetic counseling and testing for hereditary colorectal cancers may reflect a motivation to gain knowledge about the cause of cancer in one's family as well as a desire to gain information that may benefit one's family members. Studies have revealed that certain clinical factors (having a personal history of cancer or having a greater number of cancer-affected relatives) as well as psychological factors (greater perceived risk of developing cancer) are associated with greater uptake of genetic testing for mutations that cause Lynch syndrome. Less is known about the reasons why people decline genetic testing as well as the long-term consequences of these decisions, since decliners may not maintain contact with genetic counselors or other providers.

Decision aids may facilitate education and decision making about cancer genetic testing and risk management options, and may improve the efficiency of the genetic counseling process [18, 19]**.** Decision aids have been developed using a variety of formats, ranging from booklets [20] to personalized, interactive computer technology [19]. Research is emerging on the utility of decision aids for hereditary colorectal cancers. One study showed that a decision aid for Lynch syndrome genetic testing, in booklet format, was effective in reducing uncertainty about the testing decision and in assisting persons with making an informed choice about testing, and improved testing knowledge among men; however, the decision aid did not appear to influence actual testing decisions [21]. Future research should continue to evaluate the efficacy of decision aids as adjuncts to standard counseling, particularly as genetic testing is integrated into the primary care setting where access to genetics specialists may be limited.

33.2 Psychological Effects of Genetic Counseling and Testing for Lynch Syndrome and FAP

When genetic testing for Lynch syndrome and FAP was introduced into clinical practice, a primary concern was whether, and to what extent, individuals would experience adverse psychological consequences as a result of undergoing counseling and testing. Clinicians and researchers also sought to characterize those persons most vulnerable to experiencing negative effects, in order to identify specific needs for psychological support during the counseling and testing process. Studies evaluating the impact of genetic testing for hereditary colorectal cancer have focused on psychological distress outcomes (most commonly, depression, anxiety, and cancer-specific worries or distress) before genetic counseling, after counseling, and for various lengths of time after disclosure of mutation status. Responses to genetic testing have been evaluated in terms of test result (e.g., mutation-positive, mutation-negative, and inconclusive/uninformative results) as well as cancer-affected vs. unaffected status.

33.2.1 Lynch Syndrome

Longitudinal studies of psychological outcomes after genetic testing for Lynch syndrome showed that, relative to their pretest assessments, mutation carriers experienced increased general distress [22, 23], cancer-specific distress, [24] or cancer-related worry [23] immediately following disclosure of their mutation status (e.g., 2 weeks to 1 month). Compared with non-carriers, post-disclosure distress levels among carriers' were often significantly higher [22–25]. In most cases, increases in distress experienced by carriers were of relatively short duration and generally returned to pretest distress levels during the course of the year after disclosure [22–25]. Non-carriers, on the other hand, may derive psychological benefit from testing, as they generally experienced a reduction or no change in distress up to a year after results disclosure [22–25]. There are fewer data about the long-term psychological impact of genetic counseling and testing for Lynch syndrome beyond 1 year after disclosure of mutation carrier status. One study showed that at 3 years after test result disclosure, both carriers' and non-carriers' distress levels were similar to levels observed prior to genetic testing, with one exception: non-carriers' cancer-specific distress scores were significantly lower compared with their baseline scores and with carriers' scores at 1 year post-disclosure, with a similar trend observed at 3 years post-testing. [25]

Certain individuals may be at higher risk of psychological distress after notification of mutation carrier status, such as those who present with relatively higher scores on measures of general or cancer-specific distress before undergoing testing [8, 23, 26–28]. In a sample of colorectal cancer patients who had donated blood for MMR mutation testing, higher levels of depressive symptoms and/or anxiety were found among women, younger persons, and nonwhites, as well as those with less

formal education and fewer and less satisfactory sources of social support [28]. A subgroup of individuals from the same population who showed higher levels of psychological distress and lower quality of life and social support was more likely to worry about finding out that they were MMR mutation carriers and about being able to cope with their test results [29]. In a follow-up report examining responses to the disclosure of MMR test results among both colorectal cancer patients and their at-risk relatives, a subgroup with the same psychosocial characteristics reported higher levels of general distress and distress specific to the experience of having genetic testing within the year after disclosure, regardless of mutation status [23]. Other studies also have found that a prior history of major or minor depression, higher pre-test levels of cancer-specific distress, having a greater number of cancer-affected first degree relatives, greater grief reactions, and greater emotional illness-related representations predicted higher levels of distress from 1 to 6 months after disclosure of test results [27, 30]. Taken together, these findings underscore the importance of identifying persons who may be at risk for increased distress during the genetic counseling and testing process and who may benefit from psychological support and follow-up during this process [31].

33.2.2 FAP

Similar to Lynch syndrome, carriers of *APC* gene mutations may experience increased distress after notification of their genetic risk status. In a cross-sectional study of adults who had previously undergone *APC* genetic testing, mutation carriers reported higher levels of state anxiety than non-carriers and were more likely to report clinically significant anxiety levels [32]. Lower optimism and lower self-esteem were associated with higher anxiety in this study, [32] and FAP-related distress, perceived seriousness of FAP, and belief in the accuracy of genetic testing has been associated with higher anxiety among carriers in another study. [33]

Given the ongoing challenges in coping with this condition, persons with FAP can benefit from psychological support or assistance beyond the genetic counseling and testing process as well. Young adults with FAP reported needing support or assistance with the following syndrome-related issues: anxiety regarding their children's risk of developing FAP; fear about developing cancer; and uncertainty about the long-term impact of FAP [13]. In the same study, 16% perceived some form of FAP-related discrimination, mainly concerning negative attitudes toward their medical or self-care needs (e.g., time off work for screening, need for frequent toilet breaks, and physical limitations). As individuals cope with FAP throughout their lifespan, additional manifestations of this syndrome may present significant medical, economic, and psychosocial challenges. An example is desmoid tumors, which may affect approximately 10% of FAP-affected adults. Desmoids commonly occur in the abdomen and are benign; however, because treatment may not be consistently effective, these tumors can continue to grow and become life-threatening. In a sample of FAP-affected adults who also had been diagnosed with desmoid tumor,

health-related quality of life was lower compared to the general population [34]. A subsample of participants in this study noted stressors related to coping with desmoids, including uncertainty about future health status, feelings of isolation, and lack of information about desmoids, treatments, and outcomes.

A particular concern is the psychological vulnerability of children who undergo *APC* genetic testing. Studies suggest that most children do not experience clinically significant psychological distress as a result of *APC* testing. As in studies involving adults, however, subgroups of children may be vulnerable to increased distress and may benefit from psychological support during and/or after the counseling and testing process. Among children who had undergone *APC* genetic testing, mean scores on measures of mood and behavior were generally within normal ranges after genetic counseling and disclosure of test results. However, *APC* mutation-positive children with an ill mother reported significantly higher depression scores, suggesting that mothers' reactions to their own illness may strongly influence their children's reactions [35]. In a long-term follow-up study (25–55 months) of 48 *APC*-tested children and their parents, characteristics of the family context influenced psychological outcomes. Children at greatest risk of experiencing distress were those who were *APC* mutation-positive and had siblings who also were mutation carriers. Parents also are vulnerable to increased distress as their children undergo genetic testing, regardless of whether they themselves are affected with FAP. In families that included both *APC* mutation-positive children and mutation-negative children, the unaffected parents had significantly increased depression scores after disclosure of children's test results [36]. Another study found that although the perceived risk of developing the disease increased in *APC* mutation–positive children after disclosure of results, anxiety and depression levels remain unchanged in the year following disclosure [32]. Mutation-negative children in this study experienced less anxiety and improved self-esteem over this same time period.

33.3 Family Communication About Genetic Testing and Inherited Cancer Risk

Genetic testing for inherited cancer predisposition provides information about the individual tested as well as his or her biological relatives. Individuals who undergo testing (particularly index cases, or the first person tested in the family) are the gatekeepers for this information in their families [37]. Communication about genetic risk information within families is recognized as being largely the responsibility of family members, rather than health care providers. The American Society for Clinical Oncology (ASCO) has advised that health care providers educate persons who undergo genetic testing for inherited cancer susceptibility about the importance of communicating test results to family members [38]. Research on family communication about genetic testing has shown that persons generally are willing to share their test results with at least some of their relatives, often within a few weeks after disclosure [39–41]. Typically, persons are more likely to share test results with first-degree

relatives (e.g., siblings, children) rather than with more distant relatives [39–41]. Motivations for sharing genetic risk information include a desire to increase family awareness about health care options and predictive genetic testing, as well as a perceived moral obligation and responsibility to help others in the family [39, 40].

While most persons studied typically view communication about genetic risk as an open process, there may be barriers to doing so. Reasons for not informing a relative about genetic test results included lack of a close relationship and lack of contact with that person; in fact, emotional rather than relational closeness seemed to be a more important determinant of the degree of risk communication. Disclosure seemed less likely if at-risk individuals were considered too young to receive the information (i.e., children), if information about the hereditary cancer risk had previously created conflict in the family [40], or if it was assumed that relatives would be uninterested in information about testing [39]. A history of conflict in family relationships can inhibit discussions about hereditary cancer risk, particularly if such discussions involved disclosure of bad news [40].

Probands may feel particularly obliged to inform family members about a hereditary cancer risk [40] and may be strong advocates for encouraging their family members to undergo genetic counseling and testing for the cancer-predisposing mutation identified in the family [41]. Mutation-negative individuals, persons who chose not to be tested, and spouses of at-risk persons may not feel as personally involved with the risk communication process compared with probands and other at-risk persons who had undergone genetic testing [41]. Families who are more comfortable and open with cancer-related discussions in turn may be more receptive and accepting of news about genetic risk [40].

Various modes of communication (e.g., in-person, telephone, or written contact) are typically used to disclose genetic risk information within families [39–41]. In one study, communication aids such as a genetic counseling summary letter or Lynch syndrome booklet were viewed as helpful adjuncts to the communication process but were not considered central or necessary to its success [39]. Studies have suggested that recommendations by health care providers to inform relatives about hereditary cancer risk may encourage communication about Lynch syndrome [40] and that support by health care professionals may be helpful in overcoming barriers to communicating such information to family members [42].

33.4 Adoption of Risk Management Recommendations for Lynch Syndrome and FAP

Carriers of *APC* or MMR gene mutations are advised to follow risk management recommendations for colorectal and other syndrome-related cancers that address screening needs as well as preventive surgery [43]**.** A primary goal of genetic testing is ultimately to reduce cancer morbidity and mortality in families with Lynch syndrome and FAP. Thus, it is important for clinicians to understand why individuals

do or do not follow risk management recommendations, barriers to adoption of recommendations, and the effects on quality of life and psychological adjustment.

33.4.1 Lynch Syndrome

33.4.1.1 Colorectal Cancer Screening

Persons at risk for Lynch syndrome are advised to undergo colonoscopy every 1–2 years beginning at age 20–25 years [9]**.** Several longitudinal studies have compared use of screening colonoscopy by cancer-unaffected persons before and up to 1 year after Lynch syndrome genetic counseling and testing. [44–47] One study reported that MMR mutation carriers were more likely to have a colonoscopy compared with both non-carriers and those who declined testing (73% vs. 16% vs. 22%). In the year after disclosure of genetic test results, 53–73% of carriers had had a colonoscopy, and screening rates either increased or were similar compared to rates prior to testing. A study conducted in Belgium reported 100% adherence to colonoscopy among mutation carriers during the same time period [44]. Among non-carriers, colonoscopy rates decreased in the year following notification of genetic test results [45–47]. Persons who were most likely to have a colonoscopy within 1 year after results disclosure also were positive for MMR gene mutation carrier status, [44–47] were of older age, [46] and expressed greater perceived control over colorectal cancer.

Studies examining colorectal screening behaviors beyond 1 year post-disclosure of genetic test results have been conducted in Europe and Australia, and findings suggest that improvements in colorectal screening behaviors among MMR gene mutation carriers may be maintained over a longer period of time. In studies with follow-up periods ranging from 1 to 18 years after genetic testing and/or risk counseling, adherence to colorectal screening ranged from 73 to 100% among persons with a genetic or clinical diagnosis of Lynch syndrome [25, 48, 49]. Factors associated with screening non-adherence included greater perceived barriers to screening and greater embarrassment regarding colorectal screening procedures [49]. Findings from one study suggest that MMR mutation carriers may have positive attitudes toward undergoing colorectal screening in the future, as 94% of the sample stated an intention to have annual or biannual colonoscopy in the future [44]. Sixty-four percent of non-carriers in the same study indicated that they did not intend to have colonoscopy in the future or were unsure; this finding may suggest that particular attention needs to be given to counseling non-carriers about appropriate screening recommendations [44].

Taken together, these early studies indicate that genetic testing for Lynch syndrome may motivate individuals to maintain or improve recommended colorectal screening, which is a desired outcome of genetic testing for inherited cancer risk. However, screening rates observed in these studies are often less than optimal, particularly in U.S. samples, and further research is needed to identify barriers to screening as well as to develop and evaluate interventions that encourage

colonoscopy use among persons who screen less frequently than recommended. It also is important to consider how methodological features of these studies, as well as health care system differences among various countries represented, may influence screening behavior outcomes.

An issue that warrants further study concerns persons who undergo colonoscopy more frequently than the guidelines recommend. Hadley et al. [46] reported that 35% of MMR gene mutation carriers and 13% of non-carriers were classified as non-adherent to recommendations for colorectal screening within the year after disclosure of genetic test results; in both groups, about half screened more frequently than recommended and were considered hyper-vigilant. Hyper-vigilant screening behavior may be attributed to inappropriate screening advice by health care providers, or may be motivated by persistent worry about colorectal cancer risk even in light of negative MMR gene mutation results. There is a need to better understand why people may undergo colorectal screening more often than recommended, as this may result in higher health care costs, overutilization of limited health care resources, and unnecessary patient risks.

33.4.2 Gynecologic Cancer Screening

Gynecologic cancer risk management recommendations for Lynch syndrome include the option of annual endometrial biopsy with TVU for women with a suspected or documented MMR mutation beginning at age 30–35 years [9]; however, the efficacy of these strategies has not been proven. A small number of studies have examined adherence to endometrial screening in Lynch syndrome and include small numbers of women at risk. Data indicate that female mutation carriers do not universally adopt intensive gynecologic cancer screening. However, like colorectal screening, use of gynecologic screening appears to increase in response to the notification that one is at increased genetic risk for endometrial cancer. A cross-sectional study of persons surveyed 6 months to 9 years after genetic testing for Lynch syndrome found that 69% of mutation-positive women reported following gynecologic screening advice, significantly more than had done so prior to testing (10%); however, the screening interval and specific gynecologic tests were not described [49]. Among women enrolled in a Lynch syndrome registry who had received genetic counseling and risk assessment with or without genetic testing, 69% had undergone at least one endometrial biopsy [50]. Other studies have reported that within one to 3 years after disclosure of test results, 53–54% of carriers underwent endometrial biopsy and 47–86% underwent TVU [25, 44, 45].

33.4.3 Risk-Reducing Surgery

Evidence is lacking for or against the recommendation of preventive colectomy for unaffected persons with Lynch syndrome. At-risk individuals generally do not seem

to opt for this surgery as a risk reduction option, and little is known about decision making and the psychological impact of undergoing risk-reducing colectomy for Lynch syndrome [25, 45]. There also are limited data regarding the prevalence of risk-reducing hysterectomy (RRH) or risk-reducing salpingo-oophorectomy (RRSO) among women with Lynch syndrome. In one study, 69% of women reported considering RRH and RRSO prior to receiving their MMR gene mutation results, suggesting that consideration of risk-reducing surgery may have motivated interest in testing [51]. Among persons who received positive MMR gene mutation results in this study, a greater proportion indicated interest in risk-reducing colectomy compared with their baseline assessment; however, this study did not assess actual surgical decisions [51]. In a longitudinal study of cancer-unaffected persons who underwent genetic testing for Lynch syndrome, 5% of women indicated that they would have an RRH and an RRSO if they were found to be mutation-positive [25, 45]. At 3 years following disclosure of results, two women (of 13 female mutation carriers) who had undergone a RRH before genetic testing underwent RRSO within 1 year after testing, but risk-reducing surgery was not elected by any other female mutation carriers [25]. The relatively low uptake of RRH and RRSO among women with Lynch syndrome may reflect individual preferences, such as delayed decision making about surgery until childbearing has been completed. With the availability of recently published evidence showing the efficacy of hysterectomy and oophorectomy in reducing the occurrence of endometrial and ovarian cancers for Lynch syndrome [52], more women may be advised to consider these surgical options in the future.

33.4.4 FAP

33.4.4.1 Colorectal Screening

Persons at risk for FAP are advised to undergo routine endoscopic surveillance beginning at age 10 years. The limited data on uptake of colorectal screening in FAP is derived primarily from cross-sectional studies. In a small study of individuals aged 17 years and older with a family history of FAP who were offered participation in a genetic counseling and testing protocol, all asymptomatic persons had previously undergone at least one screening endoscopy but only about one-third adhered to screening at recommended intervals [53]. In this study, nearly all FAP-affected persons who had had a colectomy were adherent to recommended colorectal surveillance. In a cross-sectional study comprising persons with a clinical or genetic diagnosis of FAP or attenuated FAP and their at-risk relatives, 52% of those with FAP and 46% of at-risk relatives had undergone recommended endoscopic screening [54]. Among those who were affected by or at risk for attenuated FAP, 58 and 33%, respectively, had undergone screening. Persons who had not screened were less likely to recall receiving screening recommendations from a health care provider, more likely to lack health insurance or insurance reimbursement for screening, and more likely to believe that they were not at increased risk for colorectal cancer. A small percentage of participants (14–19%) described screening as a “necessary

evil" and indicated a dislike for the bowel preparation and/or experiencing pain and discomfort from the procedure. Similar proportions of respondents indicated that such issues may pose barriers to undergoing future endoscopies and that improvements in endoscopy and anesthesia increased tolerability of the procedures.

The desire to avoid endoscopic screening has been suggested as a motivating factor to undergo *APC* genetic testing [16]. On the other hand, one study indicated that 42% of persons who had undergone *APC* mutation testing expected to continue having endoscopic surveillance, despite the fact that their genetic test result was mutation-negative. Hypervigilance regarding screening in this study was attributed primarily to doubts about the accuracy of genetic testing, which suggests that there may be a benefit to exploring personal perceptions about genetic testing for FAP and their influence on health and screening behaviors [33].

33.4.4.2 Risk Reducing Surgery

Prevention of colorectal cancer in FAP can be achieved only through prophylactic colectomy, which is offered when polyps become too numerous to manage endoscopically, typically between the ages of 15 and 25 years. The psychosocial impact of FAP has been examined primarily through quality of life assessments after risk-reducing colectomy. General measures of quality of life have been within normal ranges after surgery, and most have reported no negative impact on their body image [55]. However, others have reported that quality of life may be negatively impacted by problems with bowel function following surgery, such as increased stool frequency, occasional liquid soiling, and worries about incontinence [56, 57].

33.5 Implications for Clinical Practice and Future Research Directions

The increasing use of predictive genetic testing for hereditary cancer risk in clinical practice has brought about rapid changes in the care of both patients as well as their at-risk family members. Progress in clinical cancer genetics over the past decade or so has resulted in both medical as well as psychosocial benefits for families with hereditary cancers. Through genetic testing, these persons have the opportunity to resolve uncertainty about their personal and familial cancer risks and can obtain information to guide and personalize decisions about their future health care. Whether or not individuals engage in recommended strategies to reduce or manage their cancer risk is critical to the ultimate translation of genetic information into reductions in cancer morbidity and mortality. Future research should continue to explore both the short- and long-term psychological impact of genetic testing and genetic risk notification and factors that influence the adoption of risk reduction recommendations at both the individual and family level. Such efforts will address current gaps in knowledge, and inform strategies for facilitating the optimal delivery of clinical services for high risk populations.

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Part XI
Chemoprevention

Chapter 34
Chemoprevention for Inherited Colorectal Cancer

Patrick M. Lynch

Abstract Before the responsible susceptibility loci were discovered in the late 1980s and early 1990s, management of Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) were based on endoscopic surveillance of individuals whose parents had already manifested adenomatosis or cancer. In best case scenarios, early progression of adenomas in FAP or early cancer in HNPCC, "curative" surgery would be performed, though cancer risk remained in any remaining colorectal segment and in the UGI tract. With discovery of the *APC* gene for FAP and the mismatch repair (MMR) genes for HNPCC, a more targeted approach became possible, with attention being limited to proven carriers. This encouraged earlier surveillance and, along with improved endoscopic management, enabled some patients to be followed conservatively for a period of time before prophylactic surgery was performed.

At about the same time, development of medical measures, as opposed to mechanical (surgical and endoscopic) interventions began to take shape. Early dietary and micronutrient interventions have largely been disappointing. Nonsteroidal anti-inflammatory agents (NSAIDs) are currently the mainstay of medical management, with sulindac the most widely used. Due to the well-known side effects of traditional NSAIDs, selective COX-2 inhibitors have been studied rather extensively. Celecoxib has shown benefit in regressing colorectal adenomas and appears to have some favorable duodenal activity as well. Cardiovascular risks of both selective and nonselective COX-2 inhibitors provide a note of caution, though the balance of risks and benefits in this high-risk population differs from that in sporadic adenoma patients in whom the issue of cardiovascular risk was first raised. Because complete, durable responses are rare, the current and projected clinical trials are focusing on new agents and combinations of agents.

Often overlooked has been the evolution of principles to guide the conduct of clinical chemoprevention trials. In this chapter, we will summarize the experience with chemoprevention in FAP and HNPCC. Critical attention will be devoted to those issues of trial design that determine the propriety of existing treatment recommendations.

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34.1 Introduction

Colorectal cancer continues to be one of the leading causes of cancer death in the US and Western Europe [1]. Colorectal cancer risk can be reduced by means of colonoscopic polypectomy [2]. These screening and prevention approaches have been widely advocated in the form of practice guidelines adopted by a number of organizations, including those whose attention is directed in whole or in part to familial predisposition syndromes [3–7], summarized by P. Lynch in 2007. These improvements in screening measures in recent years leave only limitations in attitudes and economics as barriers to significant reduction in mortality. There certainly has been less success in development of measures for treating advanced disease, with fairly modest gains in survival through the adoption of newer chemotherapy drugs and aggressive surgery.

Reasonable consensus exists regarding the prophylactic surgical approaches to the colon in FAP. Colectomy is commonly performed at the time of diagnosis of adenomas. However, it is often possible to merely follow the colon of patients in their teens for several years with periodic lower GI tract endoscopy until such time as the young subject can be a more active participant in his or her decision-making regarding surgery. As will be seen later, this interval provides one window of opportunity for chemoprevention trials.

Depending on the rectal adenoma burden, the patient may require proctectomy at the time of colectomy, ideally with construction of an ileal J-pouch anal anastomosis (IPAA). Considerable variation exists among colorectal surgeons in terms of threshold for performing proctectomy. A heavy rectal polyp burden (and certain *APC* mutation genotypes) may predict a high risk of subsequent rectal cancer or need for completion proctectomy if only a colectomy with ileorectal anastomosis (IRA) is performed. Nevertheless, many surgeons express concern regarding functional outcomes, including especially sexual dysfunction in young adults, in addition to potentially greater problems of diarrhea and seepage following IPAA. This is associated with some conservatism that leads to the performance of colectomy with IRA in patients with a range of rectal polyp burdens. This population, with persistent and recurrent adenomas of the rectum, has always been a source of subjects for chemoprevention trials. Even subjects that have undergone IPAA are at risk of adenomas and cancer of the ileorectal transition area, the length of which varies from virtually zero to several centimeters, as well as of the ileal pouch proper, though the risk is much less than to the native rectum.

Variations on the Whipple pancreaticoduodenectomy may be done on the subset of FAP patients with progressive duodenal or periampullary adenomatosis. However, surgery carries risks of short-term and long-term complication and is not commonly performed during the difficult teen years. A therapeutic alternative that would avoid or delay major surgery is a holy grail. Endoscopic measures are ever

more aggressive but cannot address the field defect that is the gut mucosa of the FAP patient. As will be seen, a variety of approaches to chemoprevention have been tested in FAP.

Chemoprevention is a term coined by Sporn to describe a pharmaceutical approach to the primary prevention of cancer. Since the early days of chemotherapy for established cancers, greater and greater emphasis has been placed upon mechanistic approaches, taking advantage of progress in characterizing molecular pathways of carcinogenesis. Meanwhile, certain precursor states have come to be regarded as model systems in which to test new agents that were tested with or without interruption of specific biochemical pathways in mind. Inherited cancer susceptibility states have provided excellent models for the testing of promising drugs. The intent here has been to reduce the risk of cancer, and to avoid or at least delay the need for therapeutic and prophylactic surgical interventions. In addition to expanding the treatment options for the conditions themselves, relatively small trials in conditions such as FAP have provided a foundation, a rationale for conducting larger-scale trials in corresponding nonfamilial or sporadic disease. For several of the susceptibility syndromes to be discussed, excellent animal models exist in which preliminary proof of principle studies have been conducted.

In the area of gastrointestinal cancer, Familial Adenomatous Polyposis (FAP) has been the susceptibility model that has been most extensively evaluated. It exemplifies the adenoma–carcinoma sequence in several important respects and lends itself well to clinical trials. Subjects with FAP have a high, relatively predictable adenoma burden. If identified clinically before cancer supervenes, there is often a window of several years between diagnosis of adenomas and the need for surgery (due to progression in adenoma size, count, or dysplasia, or because a patient has met a social target, such as completion of high school). During this window, there exists an opportunity to participate in clinical chemoprevention trials. In other instances, the subject may have already undergone colectomy, but the duodenum remains at risk of adenomatosis, as does the rectum if a proctectomy has not been performed. Because such patients tend to be older and the threshold for considering surgery higher, they may be better study candidates than subjects with intact colons (Table 34.1).

Table 34.1 Advantages and disadvantages of Familial Adenomatous Polyposis as a model for the evaluation of chemopreventive agents

Setting	Advantages	Disadvantages
Intact colon	Ideal for attenuated FAP	Often pediatric, thus ethical issues
	Most physiologic	Technically more difficult
		More expensive
		Sedation usually needed
Rectum, post colectomy	Technically easy, inexpensive, requires no sedation	Tends to include subjects with either very heavy or very light adenoma burden
Duodenum	Can be combined with assessment of colon/rectum	Passage of duodenoscope more difficult
	Area of need, as adverse effects of surgery greater than for (procto)colectomy	Polyps harder to characterize

Table 34.2 Features of FAP and "Sporadic" adenomas as related to design of clinical trials

Factor	FAP	Sporadic
Time course	3–12 months	1–3 years
Evaluation	Sigmoidoscopy	Colonoscopy
Measure	Regress quantifiable polyp count	Recurrence of polyps
Toxicity OK?	Somewhat	No
Clinical alternative	Often surgery	Continue usual F/U
Subject motivation	High	Variable
Sample size	Small (10–100)	Often >1,000

It is important to consider some of the key differences between the conduct of chemoprevention trials in a very high-risk group such as FAP and the performance of a trial in nonfamilial or sporadic adenomas (Table 34.2).

34.1.1 Early FAP Trials

In the following paragraphs, we will summarize the early clinical chemoprevention trials in FAP. Emphasis will be given to methods for subject selection, treatment schema, polyp measurement, and analytic approach.

One of the earliest attempts at chemoprevention was that of Bussey et al. [8]. In this trial conducted in the late 1970s, the retained rectums were followed in 49 FAP patients post colectomy with ileorectal anastomosis. Examinations were done over 15–24 months at 3-month intervals by means of rigid proctoscopy. Subjects were randomized to ascorbate 3 g/day or placebo, and both investigators and subjects were blinded as to treatment assignment. The number and surface area of polyps were measured. Average polyp count at baseline was 12, with 88% 3 mm or smaller. The average age at study entry was 42 years. In all, 19 ascorbate and 17 placebo subjects were evaluable, with subjects excluded on the basis of no polyps at baseline (only 2), dropout, and noncompliance. A significant treatment difference favoring the ascorbate arm was seen, but only at the 9-month measurement.

Among notable issues raised in this study was polyp measurement itself. Polyp size was estimated "by comparison to internal diameter of the (rigid) instrument". Interobserver variation in polyp counting was carried out by means of tandem or back-to-back examination by two observers in 21 cases. A coefficient of variation of 27% was found, "suggesting that interobserver variability was not unusually large", but concluding that measurement variability by one observer would be less. The biggest problem overall was the large variability in

polyp counts within patients from visit to visit, attributed to observer error and/or actual fluctuation in counts.

34.1.2 Nonsteroidal Anti-inflammatory Drugs

At about the same time as the St Marks' ascorbate trial, an ultimately more promising line of investigation with nonsteroidal anti-inflammatory drugs (NSAIDs) began with the sulindac trials conducted by Waddell [9]. Members of one family with FAP complicated by desmoids comprised the first, nonrandomized experience with NSAIDs. In the course of treating desmoids with indomethacin and sulindac, it was observed, quite anecdotally, that adenomas of the retained rectum appeared to be regressing. Indomethacin did not seem to favorably influence adenomas, so sulindac, at doses of either 75 or 150 mg, orally, twice a day, was the regimen. Four members of the same family were ultimately treated. Three with previous colectomy and IRA and one with intact colon were treated, all with good response. Incidentally, one case of diffuse gastric polyposis, presumably fundic gland polyposis, showed no response in the face of good regression of rectal adenomas.

Following the small, uncontrolled study by Waddell, several randomized, placebo-controlled trials of sulindac were conducted.

In an interesting cross-over design, LaBayle et al. [10], evaluated ten FAP patients with previous colectomy and evaluable residual/recurrent rectal adenomas. The average age of subjects was 37 years and eight of the ten were men. An initial treatment interval of 4 months was followed by a 1 month washout and then another 4-month treatment involving crossover from drug (sulindac 100 mg po TID) to placebo or placebo to drug. Polyp scoring was on a scale of grade 1: no polyps; grade 2: <5 polyps; grade 3: 5–10 polyps; grade 4:11–20 polyps; grade 5: >20 polyps. For each subject, the same endoscopist performed all procedures, using flexible scopes. Of the nine evaluable for response (one was excluded for noncompliance) who began the trial on sulindac, complete regression was seen in four subjects, with one showing a decrease in burden from grade 5 to grade 2. Reemergence of polyps was noted at the end of washout and the placebo interval.

The key US trial with sulindac in FAP was that of Giardiello et al. [11].

This single institution study from Johns Hopkins University in Baltimore was a randomized, double-blind, placebo-controlled study. It evaluated 22 FAP patients, only four of whom had previously undergone colectomy with ileorectal anastomosis. The two-arm trial randomized subjects to oral sulindac, 150 mg twice a day or look-alike placebo for a period of 9 months. By sigmoidoscopic examination at 3-month intervals, the number and size of the polyps were scored. In the sulindac arm, there was a statistically significant decrease in the mean number and diameter of polyps, compared to placebo. Overall, at end of treatment (9 months), polyp

count and diameter was 44% and 35% of base-line, respectively, in sulindac-treated cases. However, none sustained complete regression of polyps. Also, at three months posttreatment, the number and the size of polyps increased, though still significantly lower than baseline. No significant adverse side effects were attributed to sulindac.

34.2 Selective COX-2 Inhibitors

Because of the various toxicities of NSAIDs, efforts have been undertaken to develop agents with the same anti-inflammatory and adenoma-inhibiting properties but lacking in side-effects. NSAIDS exert at least some of their effects through the inhibition of cyclooxygenase (COX) enzymes, involved in metabolism of arachidonic acid to prostaglandins mediating inflammation and tumorigenesis. One isoform of COX, known as COX-2, is not constitutively expressed in most tissues to any significant extent, but is induced in inflammatory states and tumors. Selective inhibitors of the COX-2 isoenzyme, such as celecoxib and rofecoxib, collectively known as COX-2 inhibitors or "coxibs", have been developed. They do avoid some unwanted side effects of COX-1 blockade that occur with nonselective NSAIDs, including inhibition of platelet aggregation and interference with mucosal protection, the basis for NSAID-associated ulceration and GI bleeding.

In animal models, knockout of the COX-2 gene reduces intestinal polyps in a mouse FAP construct [12], while inhibition of COX-2 with coxibs reduces the incidence of carcinogen-induced cancer [13]. There are many excellent recent reviews of the mechanisms of COX activity, COX inhibition, and the pros and cons of COX2 inhibition as a meaningful factor in cancer inhibition [14].

34.2.1 Clinical Trials of COX-2 Inhibitors

In a US–UK collaboration, the first FAP chemoprevention trial with a COX-2 inhibitor, celecoxib, was undertaken on 83 FAP patients, most of whom had undergone previous colectomy with IRA. In the colorectum was observed an approximately 30% reduction in adenoma counts at a dose of 800 mg/day [15]. A nonsignificant 11% reduction in adenoma burden was seen at a lower dose of 200 mg/day. Reduction in duodenal polyposis was also observed, but was less marked [16]. On the strengths of the Steinbach trial, celecoxib was approved by the US Food and Drug Administration (FDA) as an adjunct to surgical and endoscopic treatment in FAP. Since that time, a number of follow-up studies were launched in FAP and in nonfamilial or sporadic adenomas.

Rofecoxib, at a dose of 25 mg/day has been evaluated in one small clinical trial of FAP. In six Israeli subjects, the rate of new polyp formation was estimated. At baseline and on follow-up exams, all detectable rectal adenomas were ablated, with the burden of new polyps assessed at 12 and 18 months of treatment. The baseline

polyp range of 4–20 was reduced to 0–6 at 12 months and 0–6 at 18 months [17]. This interesting strategy of combined ablation and continued drug treatment is attractive as it represents the approach taken in clinical practice with subjects receiving sulindac or celecoxib.

Sulindac sulfone (exisulind) is a metabolite of the parent sulindac compound. It is unusual in that it does not inhibit COX-2. In an 18-subject pilot study, exisulind was found to regress adenomas [18]. Because of side effects at higher doses, the follow-up Phase III trial employed a lower dose. The trial was terminated early and details of its findings have not been published to date. In one of the very few trials to target duodenal adenomas in FAP, no efficacy was found with exisulind in a randomized, placebo-controlled trial at the University of Utah [19].

34.2.2 Primary Prevention of Adenomas in Children with FAP

Given the demonstrated efficacy of sulindac in inducing regression of adenomas in adults with adenomas, a logical next step was to ask whether sulindac might be at least as effective in preventing adenomas from occurring in the first place in young carriers of *APC* mutations but who had not yet developed adenomas. Following their demonstration of short-term sulindac efficacy in regressing existing adenomas, Giardiello and colleagues at Johns Hopkins University conducted a randomized, double-blind, placebo-controlled study in 41 individuals aged 8–25 years who had *APC* mutations but were as yet phenotypically unaffected [20]. Sigmoidoscopy excluded subjects with any adenomas in the distal 20 cm of colorectum. Polyp-free subjects then underwent *APC* testing. Many screenees were ineligible due to the presence of polyps or to noncarrier status. Eligible subjects were randomized to 75 or 150 mg (according to body weight) of sulindac orally, twice a day, or to placebo. Subjects were treated for up to 48 months. At follow-up sigmoidoscopy, the number and size of new colonic adenomas were scored. Side effects of therapy were documented.

Average compliance was good over the four years of treatment, overall >76% in the sulindac group. Adenomas did emerge in 9 of 21 subjects in the sulindac group (43%) and in 11 of 20 subjects in the placebo group (55%). There was no between-group significant difference in the mean number of polyps ($P=0.54$). It was concluded that sulindac did not effectively prevent the initial emergence of adenomas in FAP. However, it must be emphasized that this was a small trial. A larger sample might have yielded a significant difference between groups.

A two institution trial (MD Anderson Cancer Center and the Cleveland Clinic) completed a Phase I trial to establish a safely tolerated dose of celecoxib in children with FAP. This anticipates a larger trial in genotype-positive children age 10–17 with few or no adenomas. In the Phase I trial, no increase in adverse events was noted in a high-dose cohort (receiving 16 mg/kg/day of celecoxib, similar to the 400 mg BID dose used in adults) compared to placebo, over an interval of 3 months. In this trial, a secondary endpoint of adenoma burden was evaluated by means of colonoscopy

at the beginning and end of the trial. The 18-subject pilot contained six subjects in three cohorts, treated with escalating doses of 4, 8, and 16 mg/kg/day or placebo in a ratio of 4 drug: 2 placebo in each cohort. Because the pilot did not require an absence of polyps, adenoma regression was measurable. As in the adult trial, the high dose group did experience a significant reduction in adenoma burden, compared with those receiving placebo [21].

Proceeding from this Phase I trial, a multi-center Phase III trial of celecoxib versus placebo is getting underway, enrolling children over age 10 with *APC* mutations with few (<20) or no adenomas. The trial is otherwise somewhat similar to the Giardiello sulindac trial, but will be larger, will involve full colonoscopy, and will continue for up to 5 years of follow-up for each subject. The primary endpoint is time to treatment failure, defined as interval development of 20 or more adenomas in children that are either free of adenomas at baseline or who are rendered polyp free by means of ablation of up to 20 adenomas.

34.2.3 Combination Chemotherapy

Drug resistance is a common problem in oncology. In the area of chemoprevention of adenomas in FAP, we know that there is considerable diversity in response to commonly employed agents such as sulindac and celecoxib, with "complete response" an uncommon and rarely sustained phenomenon. As in conventional medical oncology practice, we must be prepared to exploit multiple drug pathways to overcome drug resistance.

34.2.4 The CAPP I Trial

A primarily European Union-based multi center trial compared aspirin, resistant starch, both, and neither in a factorial design in subjects with FAP [22]. The largest trial to have been conducted in FAP, its results are still unpublished. However, summary presentation of data has shown rather disappointing results, with neither the aspirin, starch, or aspirin plus starch arms showing a significant reduction in adenoma burden.

A trial nearing completion of enrollment has been conducted at the University of Texas MD Anderson Cancer Center, St Marks Hospital (London) and the Cleveland Clinic. The overall design and endoscopic protocol follow very closely that of the early celecoxib trial [15]. Because celecoxib was approved by the FDA and use of chemopreventives is considered standard of care by at least one set of clinical practice guidelines [23], use of a placebo arm was not considered ethical by the institutional review boards. The control arm, therefore, is celecoxib 800 mg/day in divided doses, with or without the addition of Difluoromethylornithine (DFMO) at a dose of 0.5 mg/kg/day. The relatively large sample size is needed

since the use of active drug as the control arm anticipates the need to detect a marked treatment effect over and above the 30% reduction in adenoma burden attributable to celecoxib alone. Enrollment to this trial was suspended for more than 6 months as part of a world-wide moratorium on use of COX-2 inhibitors following the cardiovascular adverse event signals from the rofecoxib and celecoxib sporadic adenoma trials. The trial was reopened only after imposing stricter exclusion criteria pertaining to CV risk factors.

Among key questions for the future will be: (1) the choice of agents to employ; (2) potential need for new Phase I trials to assess the toxicity of combination treatment; (3) possible need for dose adjustments; (4) decisions about whether a "standard" agent such as sulindac or celecoxib should be routinely employed as one of the agents; (5) if so, the need for power calculations to provide for the larger sample size needed to show combination treatment benefit; (6) the need for multicenter enrollment due to the larger sample size. Clearly, combination treatment will bring up many of the opportunities and challenges encountered in designing and conducting cancer chemotherapy trials.

34.3 Logistic Challenges in the Conduct of Clinical Trials in FAP

34.3.1 Endpoint Measure: Adenoma Reduction

Most trials that have been conducted to date involve attempts to induce regression or shrinkage of adenomas that are already present. In the setting of a clinical trial or even clinical practice, determining whether or not adenoma reduction has been achieved is not as simple as it might first appear. Is the goal reduction in adenoma count by complete resolution of adenomas? Is it reduction in diameter or surface area of polyps even if still present? Is it prevention of new adenomas or growth of existing ones? Whichever endpoint(s), how is it measured? Most of the time, the GI endoscopist counts adenomas detected on sigmoidoscopy or colonoscopy. There is variation in quality of bowel prep and thus ability to see portions of the mucosa. The time devoted to the "pull-back" phase of the exam may not be the same from exam to exam. When there are many (>10) polyps, it may be difficult to keep a running count of the polyp number, much less the individual diameters. If a polyp is very small and flat, it may be difficult to identify at all and hard to decide if it should be "counted" at all as it may actually be a hyperplastic polyp or lymphoid aggregate and not a true adenoma at all. Should enhanced imaging techniques such as indigo carmine spray or narrow band imaging (NBI) be employed in order to get a more "true" sense of adenoma burden? If these techniques are employed, the difficulty in accurately counting all lesions is compounded.

We are not aware of any published study purporting to validate any particular method of quantifying adenomas endoscopically. We have concluded that while

there is considerable intra- and interobserver variation in the counting of polyps, this can be addressed with suitable statistical methods. There certainly has been little attention devoted to achieving reproducibility of conditions from exam to exam over time. Rather than try to compare total polyp counts pre- and posttreatment, we have adopted a "global assessment" comparison of video images. A panel of experts reviews paired videos, the order of which are randomly shuffled, assigning scores in which video "A" is considered "worse," "same," or "better" than video "B".

Efforts have been made to achieve some possibility of oversight by capturing photographs of informative polyp clusters. Use of biopsy forceps or hinged, graduated measuring tools placed adjacent to polyps in such pictures affords an opportunity to better quantify polyp dimensions.

One or more still photographs invariably fail to capture the full adenoma burden. Videotaping entire procedures or segments of exams enables more complete data capture. When this is done, new problems emerge. In a short rectal stump, reasonable reproducibility can be achieved, assuming prep quality and exam time are carefully addressed. However, in subjects with intact colons, the endoscopist frequently must stop to irrigate and clean a region. As loops are common, the scope commonly slips as it is withdrawn, necessitating reintroduction of the scope over a variable distance. Even with videotaping, it can be very difficult to tell whether a given region has been viewed once or multiple times, and thus whether a given polyp has been counted once or more than once. Placement of an India Ink tattoo is commonly done to mark a region of interest, but it is not feasible to place multiple tattoos. While usually helpful, tattoos can be problematic. The ink can extravasate, leading to not only an immediate mess, but also the potential to fade over time, potentially being missed on follow-up. If one is attempting to review paired before- and after-treatment videos or photos without knowing which is pre- and which is posttreatment (the ideal situation), the reviewer may be biased by the recognition of an obviously fresh tattoo, placed at baseline.

Trials of adenoma prevention through the use of chemopreventive agents have recently been a source of considerable controversy. At issue have been the cardiovascular (CV) toxicities of COX-2 inhibitors. There had been no real issues of cardiovascular safety in the small, short-term FAP trials enrolling relatively young subjects – those enrolled in the Steinbach celecoxib trial averaged 37 years.

However, in the much larger sporadic adenoma trials utilizing rofecoxib and celecoxib over a period of several years, there was much greater opportunity to detect a "CV signal" if there were one. There had already existed a theoretical basis for potential cardiotoxicity [24–26]. The APPROVE trial with rofecoxib was the first to show an increase in the rates of CV events – stroke and myocardial infarction – in subjects consuming essentially standard antiarthritic doses of rofecoxib for at least 18 months, a signal that promptly led Merck to remove rofecoxib from the market. A few months later, an interim analysis of data from the APC and PreSAP celecoxib trials showed a somewhat similar result.

34.3.2 Chemoprevention in Hereditary Nonpolyposis Colorectal Cancer

Hereditary Nonpolyposis Colorectal Cancer (HNPCC), is characterized by a constellation of early onset colorectal cancer, often right-sided, multiple, and with typical but nonspecific pathology features. An autosomal dominant cancer predisposition involving mutations in the mismatch repair genes *hMSH2*, *hMLH1*, *hMSH6*, *hPMS2*, HNPCC, or Lynch syndrome includes tumors of the endometrium, extracolonic GI tract, uroepithelium, brain, and sebaceous skin neoplasms. A key epigenetic feature that has proven useful in a stepwise approach to molecular diagnostics is the typical (>90% of cases) presence of microsatellite instability (MSI) in cancers and some adenomas. MSI tumors nearly always exhibit a loss of the protein otherwise associated with one of the underlying four MMR genes, enabling immunohistologic evaluation of tumors.

The clinical features of HNPCC were sorted out much later than those of FAP, due to the absence of the striking polyposis phenotype. Indeed, while a given case of HNPCC may have a striking clinical and family presentation, just as often the diagnosis is made somewhat later in life and with a family history that is modest. Consequently, there is some controversy as to when the use of MSI and IHC evaluation should be undertaken for a particular patient.

Because these features of HNPCC are relatively less striking and more recently sorted out, relative to FAP, there is far less experience with its clinical management. Consequently, there is less agreement regarding the approach to surgical resection for cancer, with some arguing for subtotal colectomy to reduce the risk of synchronous cancers, while others support a more conservative approach involving segmental resection followed by close endoscopic surveillance. Less attention has been devoted to consideration of the proper role for endoscopic management of adenomas in HNPCC. Good, but nonrandomized data exist to show a cancer reduction and mortality benefit through the use of colonoscopy screening, beginning fairly early in life and repeated at shorter intervals than are recommended for the general population. Most of the best data are from a cohort of subjects followed in Finland by Jarvinen, Mecklin, and colleagues over the past 20 years [27–30], though series from other countries have been published [31–33].

Far fewer efforts have been undertaken to prevent adenomas in HNPCC than in FAP or even sporadic adenomas. There are excellent reasons for this being the case. Until the mismatch repair genes were identified, it was not possible to be certain which patients were actually carriers of susceptibility, excepting patients already having undergone surgery for cancer. Like FAP, HNPCC is an uncommon condition and recruiting a large sample of subjects quite challenging.

Unlike FAP, studies involving adenoma regression are not really feasible. At a given colonoscopic exam, adenomas are only marginally more common than in the general population, at least if uncorrected for age. The rapid growth of adenomas in HNPCC and the fear of high-grade dysplasia makes the monitoring of a given lesion over time somewhat hazardous.

The development of prevention trials in HNPCC suffer from many of the same limitations as regression studies, except more so. Given the rate of new adenoma formation in HNPCC, demonstration of a drug's treatment effect in causing reduction in occurrence/recurrence requires sample sizes similar to those involved in sporadic adenoma trials, in the range of 500–1,000 subjects or more.

Nevertheless, a few studies have been conducted, differing considerably in approach.

In one ostensibly biomarker-oriented study, a microarray approach was performed in order to identify genes that are overexpressed and underexpressed in HNPCC carriers in response to administration of celecoxib. This small trial of less than 80 subjects did show that celecoxib affects expression of a host of genes having potential regulatory significance [34]. However, to date no follow-up has been conducted for them.

34.3.3 CAPP II

Eagerly-awaited results of the Concerted Action Polyp Prevention II (CAPP II) trial in HNPCC have recently been published [35]. The design was rather similar to that of the CAPP I trial in FAP in that the agents employed were aspirin (two 300 mg enteric coated tabs daily), resistant starch (Novelose, a 70% amylase/30% amylopectin, daily), both, or neither. The daily starch dose of about 13 g was estimated to be about three times the average daily starch consumption in Europe. Of the slightly more than 1,000 subject enrolled, 83% had a known deleterious germline mutation in an MMR gene. The remainder had a clinical diagnosis based on personal history of CRC and membership in a family meeting Amsterdam Criteria for HNPCC. Nearly a third of these subjects were later found to have MMR gene mutations. Subjects were greater than age 25 at study entry. Colonoscopy with polyp clearance was required within three months of study entry. Exclusions included recent CRC, intolerance/allergy to aspirin, severe medical comorbidities, pregnancy, or ongoing use of NSAIDs. The primary endpoint was the development of colorectal adenoma or cancer at the time of study exit colonoscopy, carried out after 2 years of intervention. About 25% dropped out either before the entry colonoscopy or prior to a follow-up colonoscopy, leaving 746 evaluable subjects. Compliance was good, with more than 80% of subjects consuming at least 80% of prescribed aspirin, with similar compliance for the resistant starch. On average, duration of study drug usage was 27 months. Subjects underwent an average of three colonoscopies in the course of the trial, including study entry.

An adenoma or CRC was found in about 19% of subjects. Over the course of the trial, no difference in rates of new neoplasm formation was noted between the active study groups (18.9%) and those receiving placebo (19.0%). There was no evidence of interaction between the study agents, aspirin and starch. "Advanced neoplasm", that is, large and/or severely dysplastic lesions are a common and important endpoint in chemoprevention trials. These showed no difference between

treatment groups either. No significant difference in adverse events was observed by treatment group, including GI ulceration and cardiovascular events.

The authors speculated on various possible causes for the disappointing lack of effect of aspirin. This included the possibility that the MMR pathway is sufficiently different from the more common APC pathway as to simply be less responsive to COX-2 inhibition, the main proposed mechanism of action for aspirin and nonaspirin NSAIDs. The relatively short duration of the trial was also considered an issue, as some data do indicate that a 10 years or more duration of use may be critical.

34.4 Conclusion

Key properties of the ideal clinical chemopreventive agent are efficacy, safety and low cost in long-term administration. If the cancer risk and related morbidity of the underlying condition are high, then some concessions may be made in one or more of these areas. In FAP, as opposed to sporadic adenoma patients, investigators, clinicians, patients, and regulatory authorities may be willing to take some greater risks. Because FAP patients are, on average, younger when the question of chemoprevention is raised, the balance may favor the use of NSAIDs when it otherwise would not. Little attention has been devoted to matters of long-term safety and efficacy. We also do not know whether use of the tacitly accepted agents sulindac and celecoxib has ever led to longer intervals of surveillance. Nor is there sufficient evidence that doses of these agents can be lowered in the interest of reducing toxicity, while at the same time maintaining efficacy. Greater attention to these matters is important. So is the need to continue looking for safer, more effective drugs and drug combinations.

Molecular advances over the past 15 years make it all the more likely that newly developed chemopreventive agents will have been extensively tested in vitro and in knockout animal models prior to use in human trials. Consequently, more and more attention to translational, multidisciplinary teamwork in new drug development and testing can be anticipated, and will be welcomed by clinical investigators.

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Part XII
Registries

Chapter 35
Registries*

Steffen Bülow and Inge Bernstein

Abstract Familial Cancer Registries have proven to be invaluable in identifying individuals at risk of hereditary colorectal cancer syndromes and their families. With the advent of registries, call-up patients shown to have better survival than index patients. In this chapter, the establishment, purpose, and function of familial cancer registries will be discussed.

Keywords Familial adenomatous polyposis • Hereditary non-polyposis colorectal cancer • Lynch syndrome • Hereditary colorectal cancer • Registry • National registration

35.1 Introduction

Familial Cancer Registers on Hereditary Colorectal Cancer are collections on demographic and disease-related data on families with inherited increased risk of colorectal cancer (CRC) with the overall objective to improve the prognosis and quality of life for the individual family members by establishing diagnosis and risk calculation during continuous update of data and research.

This goal is achieved in different ways in the different counties, as some registers are national and some regional, some take direct part in patients care and some are designed for research only. The tradition for establishment of registers and the exact data content as well as the legal possibilities to obtain information from population registers, parish registers, cancer registers, and probate courts, if such exists, differs greatly in the different regions and countries.

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35.2 History

In 1925, Lockhart-Mummery described three families with familial adenomatous polyposis (FAP) and underlined the hereditary nature, the tendency to cancer development, and the importance of examination of close relatives. These families became the nucleus of the first polyposis register in the world at St. Mark's Hospital [1]. Since the 1970s, national polyposis registries have been established in several European countries: Sweden, Denmark, Norway, Finland, the Netherlands, Belgium, and Lithuania, and a large number of regional registries have emerged all around the world [1–135] (Fig. 35.1, Table 35.1).

A similar history exists for the development of HNPCC registers worldwide, where the initial description of the famous Family G was published in 1913 by Warthin [54] and later followed up by Lynch [55]. It was not until the establishments of registers in Omaha, Finland and the Netherlands [56–58] in the 1980s, however, that important research became possible and the natural history of the syndrome was understood. Based upon this initial important work, registers have been established in many countries as registration obviously is a prerequisite for adequate management and research.

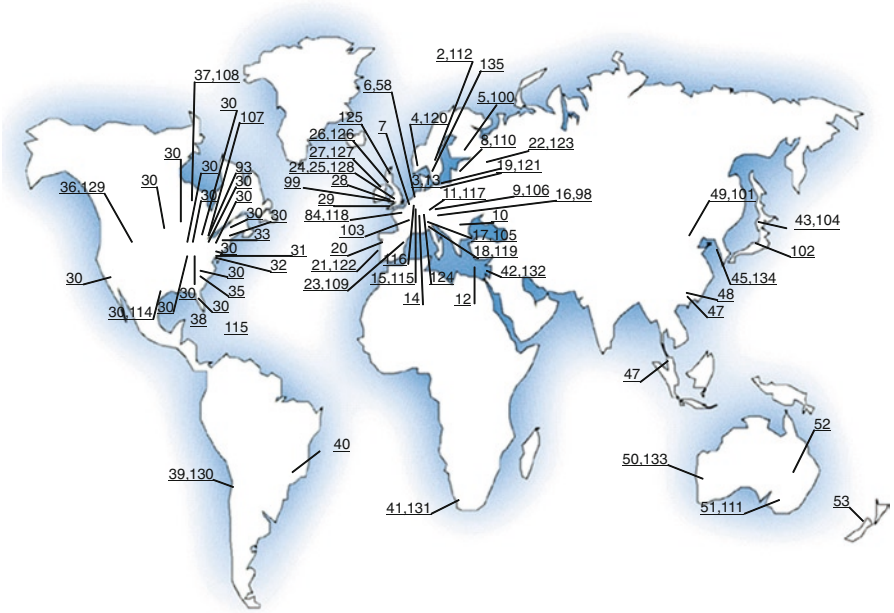

Fig. 35.1 National polyposis registries globally

35.3 Aim

The superior aim of a polyposis or HNPCC register is to improve the prognosis of the disease through identification of high-risk families, who would benefit from screening, construction of the pedigrees and the subsequent prophylactic examination of family members at risk, leading to early diagnosis of affected individuals and subsequent cancer prophylactic treatment.

Additional aims are to coordinate surveillance and information on the syndrome to patients, family members and colleagues, collect results of the established screening procedures in order to adjust programs according to risk and cost-effectiveness analysis and to perform local research and participate in international collaboration.

35.4 Roles

35.4.1 Clinical Function

The register should build and maintain a detailed and continuously updated database including demography, family pedigrees, clinical manifestations, molecular genetic and clinical diagnosis, natural course, endoscopic and surgical treatment and follow-up concerning all members of the families, including affected members as well as those at risk. Data are stored in a standardized format, which will allow the daily use as well as analysis for research purposes. The register coordinates and facilitates genetical and endoscopic surveillance, genetic counselling, surgical treatment and follow-up in cooperation with molecular genetic laboratories and clinical departments. Furthermore, the register has an important role in offering counselling and support to the patients and family members regarding, for example, genetical and medical problems or insurance problems. A link to a patient society may prove a very successful source of information and encouragement. Exchange of data between different actors participating in the diagnosis and treatment of the specific family, where members often are living in different geographic places, is necessary to avoid expensive double investigations and misconception due to various information on the disease, cancer risk and surveillance given to the individual family members.

35.4.2 Education

The register provides detailed information to patients and health care professionals on all aspects of the disease, including the way syndrome is inherited, the risk of cancer, and the pros and cons of the different endoscopic and surgical methods. In this way, the patients will be prepared for the informed consent to the procedures of examination and treatment. Information leaflets, regular newsletters, information

Table 35.1 Polyposis and HNPCC registries and centres

	Country	Region	References
Europe	Austria	Vienna	[9, 106]
	Belgium	National	[7]
	Bulgaria	Sofia	[10]
	Czechia	Prague	[11, 117]
	Cyprus	Nicosia	[12]
	Denmark	National	[3, 13]
	Finland	National	[5, 100]
	France	Paris	[84, 118]
		Lyon	[103]
	Germany	Heidelberg	[14]
		Bonn	[15, 115]
		Düsseldorf	[116]
	Holland	National	[6, 58]
	Hungary	Budapest	[16, 98]
	Italy	Modena	[17, 105]
		Milan	[18, 119]
	Lithuania	National	[8, 110]
	Norway	National, Oslo	[4, 120]
	Poland	Poznan	[19]
		Szczecin	[121]
	Portugal	Coimbra	[20]
		Lisbon	[21, 122]
	Russia	Moscow	[22, 123]
	Spain	Mallorca	[23, 109]
	Sweden	National	[2, 112]
		Lund	[135]
	Switzerland	Basel	[124]
	UK	St. Mark's Hospital	[24]
		Thames Region	[25, 128]
		Newcastle upon Tyne	[26, 126]
		Northern Ireland	[27, 127]
		Birmingham	[28]
		Cardiff	[29]
		Oxford	[99]
		Edinburg	[125]
North America	USA	California	[30]
		Buffalo	[93]
		Delaware	[31]
		Florida	[30]
		Houston	[114]
		Illinois	[30]
		Indiana	[30]
		Kentucky	[30]
		Maryland	[32]
		Massachusetts	[30]
		Missouri	[30]

(continued)

Table 35.1 (continued)

	Country	Region	References
		Michigan	[30]
		Minnesota	[30]
		Nebraska	[30]
		New Jersey	[30]
		New York	[33]
		North Carolina	[30]
		Ohio	[30]
		Pennsylvania	[30]
		South Carolina	[36]
		Texas	[30, 114]
	Puerto Ricco National [113]	Utah	[36, 129]
		Vermont	[30]
	Canada	Ontario	[37, 108]
		Toronto	[107]
	Cuba	Havana	[38]
South America	Chile	Santiago	[39, 130]
	Brazil	Sao Paulo	[40]
Africa	South Africa	Cape Town	[41, 131]
Asia	Israel	National	[42, 132]
	Japan	Tokyo	[43, 104]
		Hiroshima	[102]
		National	[44]
	Korea	National	[45, 134]
	Singapore	National	[46]
	Hong Kong	Hong Kong	[47]
	China	Guangdong	[48]
		Beijing	[49, 101]
Australia		Western Australia	[50, 133]
		Victoria	[51, 111]
		New South Wales	[52]
New Zealand		National	[53]

videos and websites may also contribute to raise the informational level of the patients and of the general physicians too. The register staff should be continuously updated about the recent literature and thereby be able to answer questions on new developments in the field.

35.4.3 Research

The register is not only an important data source in several fields of medicine involved in the professional healthcare of the individual patient and the family, but also an important tool in the decision-making process on priorities in the public healthcare system.

In general, the registries are voluntary in nature and they can only function in an atmosphere of mutual trust between the registries and the family members.

35.5 Type of Register

Registries operate alongside, but often outside the conventional medical system, and the organizational conditions are often the result of pioneers' effort and enthusiasm, some placed in the surgical departments, some in clinical or molecular genetic departments. There are three types of registries:

National registries have been established in countries with rather small and stable populations, a well-recorded healthcare system and a centralized recording of all citizens, most often in Northern Europe: Denmark, Sweden, Norway, Finland, the Netherlands, Belgium and Lithuania, but also in Singapore and Japan. The registries collect data from the hospitals and have links to public registers, but the register staff is rarely involved in the direct patient care. National registries provide optimal possibilities for epidemiological research concerning incidence and prevalence, and for long-term follow-up studies including survival studies along with coordination and evaluation of national screening recommendations.

Regional registries have been established in countries with large or widespread populations, for example, in the UK, USA and Germany. The function is similar to the national registries with a limited involvement in patient care.

Referral centres often include a register, which has developed due to the specific interest of experts in the disease. The patients are referred to a major centre as a difficult case, or to a specific surgeon. Secondly, the family is mapped and members at risk are being examined and treated. Examples of such registries are St. Mark's Hospital in London, The Cleveland Clinic in Ohio and Mt. Sinai Hospital in Toronto. The advantage of these centres is their large patient series, a substantial experience in rare manifestations and a long tradition of treating complex patients.

35.6 Data Confidentiality

Great care should be taken to ensure confidentiality and to protect privacy of the data due to the unique mixture of personal data and family data, which constitute the basis for diagnosis, treatment and surveillance of a genetic disease. National rules about confidentiality and data handling must be followed and each individual has the right to correction and removal of his personal data at any time. Registered individuals are informed that anonymized information may be used for research purposes, and research projects are approved by the ethics committee system and data approval system.

35.7 Establishment of a Register

Primarily, the type of register must be decided, and secondly the pattern of local expertise in the medical specialities should be assessed: surgeon, gastroenterologist, medical geneticist, molecular geneticist, and others. The funding for the daily running of the register may originate from local or national public sources, institutions, cancer societies or charity, and research projects may be funded from public or private sources. It is important to find a coordinator who is capable of organizing the daily work and update of the register as well as communicating with patients, relatives and physicians. Finally, the computer software should include a user-friendly database programme combined with a pedigree drawing programme. Nowadays, several types of commercial software are available for this purpose, and officers of the InSiGHT may assist and advise in this matter.

The establishment of a new register includes several stages [13, 59, 60, 93]:

1. Ascertainment of patients
2. Construction of pedigrees
3. Identification of relatives at risk
4. Establishment of screening of relatives at risk
5. Treatment of newly diagnosed patients
6. Follow-up and evaluation

35.7.1 Ascertainment of Patients

The register should attempt to trace patients from multiple sources. Enquiries should be made to colorectal surgeons, adult and paediatric gastroenterologists, clinical geneticists and general practitioners through national or regional medical societies. During this process, it is important to underline that the register is not planning to "take over" the patients, but only to include information about new cases. Cancer registries should be searched for colorectal cancer patients below age 50, and patient registries, parish registries, and stoma nurse services are other potential sources. The national literature should be surveyed for reports on cases, and announcements in medical papers may help to encourage referrals. Families could also be included directly by public invitation to participate through information in newspapers, television, brochures or WEB-sites.

35.7.2 Construction of Pedigrees

The *genealogical information* may originate from several sources. The family history can be built upon the basis of information from several family members over a long time and the details in the medical information should be traced and checked

to the highest documentation level. The family history may include full names, date of birth, medical history including diseases, operations, names of hospitals, date of death, etc. A contact person in each family may be of great help in this work. Additional information originates from family doctors, private specialists, parish registers, probate courts and local population registries. Some countries have a computerized central population register containing basic demographics data of all citizens, including data about the parents, siblings and children. *Medical information* is obtained from the patients, family doctors, private specialists, clinical hospital departments, copies of death certificates, autopsy reports and cancer registries. In the end, a detailed pedigree is built and often includes family members in many generations.

35.7.3 Identification of Family Members at Risk

Identification of family members at risk is a combination of pedigree analysis and possible molecular analysis (MSI, IHC, mutation detection). If molecular analysis is not an option, the risk estimation is judged alone on the phenotype in the family. In families with autosomal dominant disease, the offspring of an affected individual has a 50% risk at birth of being a gene carrier.

Patients with FAP can be diagnosed by their own phenotype with multiple adenomas in the rectum. If the characteristic phenotype does not develop, the risk of being gene carrier gradually declines by increasing age to about 5% at age 30 years, but it does not reach zero due to a small chance of non-penetrance, that is, a gene carrier does not develop the disease. New mutations in the APC-gene are responsible for probably 10–20% of all new cases of FAP, and siblings of a new mutation have about 5% risk of being affected due to gonadal mosaics. Skipped generations are not seen in FAP.

Identification of HNPCC families are more complex as this is not entirely based on the phenotype in the individual, but the phenotype of the whole family with at least documentation for HNPCC cancer in three family members or identification of a disease causing mutation in the MMR genes.

The completed pedigree of each family with additional medical information forms the basis for the identification of family members at risk, that is, first-degree relatives of affected members – or in some cases second-degree relatives if sufficient genealogical and/or medical information is not available about first-degree relatives: an individual known to be at risk may have died from an unknown cause or may refuse to be examined.

How to perform dissemination of information on risk and possible disease prevention to all relevant family members have initiated a debate in many countries, and focus has also been on the fact that genetic registers initially used for research could be used for treatment in hereditary cancer prevention [61–67]. In general, the assurance of privacy and confidentiality is considered the cornerstone of the patient–physician relationship, which easily can conflict with the doctors' duty to warn, intervene

and treat other relatives at risk. Traditionally, information is given in a genetic counselling setting to the index person with the obligation to inform other family members. It could be argued that life-threatening risk to relatives – as in hereditary colon cancer – should outweigh personal privacy and that the state should instruct the Hereditary cancer registers to promote surveillance programmes also by own initiative. This has been the case in Denmark where the minister of Health in 1995 instructed the HNPCC register to inform persons directly about their possible risk and options of prevention by using the information generated in the register [66].

35.7.4 Screening of Family Members at Risk

In families with a known disease causing mutation in the APC, MYH or MMR genes screening of family members at risk primarily includes DNA analysis to clarify gene carrier status. DNA analysis is recommended in combination with genetic counselling by a medical geneticist, who can inform the individual patients and family members about the basic rules of a hereditary precancerous disease and the pros and cons of molecular genetic analysis. Verified gene carriers and first-degree relatives at risk in families without a known mutation should start regular surveillance. The results of these procedures should be registered, not only to evaluate the outcome, but also in order to identify new persons at risk, in whom screening would be beneficial.

35.7.5 Treatment of Newly Diagnosed Patients

The register should be assisting the clinicians in the treatment decision for the individual persons at risk both on established screening program and adjustments along the time of follow-up, but also on the decision-making process for optimal surgery procedures either prophylactic or in case of cancer.

35.7.6 Follow-Up and Evaluation

At-risk persons are offered a lifelong regular surveillance and follow-up to prevent the development of cancer by removal of pre-malignancy. Collection of these data in the register is necessary for evaluation on outcome and cost-effectiveness of the established procedures and in order to provide the clinicians with the best evidence of optimal treatment and information for their patients. Exact disease-causing mutation in a given family is often unknown and the risk estimation is then based on clinical information on many family members – this estimate will change over time as new pre-malignancy or cancers develop in the family, which also might give the

option to perform molecular analysis. Continuous recording of genotype–phenotype in the families gives the possibility to estimation risk of different cancer types, metachronous CRC and interval cancer according to the individual family and established screening.

35.8 Research

Collection of data in a register not only is of great importance for the treatment of the individual patients and their family but is also crucial as a research tool. Epidemiological studies cannot be performed without well-functioning registers, where large amount of data are collected and dealt with and may registers contribute substantially in this field [42, 44, 68–80]. Several other projects in molecular genetics, clinical genetics, endoscopic and surgical treatment and follow-up results originate from register data, a necessity to gain further information on the diseases and genomics in order to support genomic-based medicine and adjust screening programmes established in families with different inherited risk of developing CRC.

International cooperation is frequently mandatory to generate sufficient data for a specific study.

35.9 International Cooperation

The majority of registers are members of InSiGHT: The International Society for Hereditary Gastrointestinal Tumours, which was established in 1999 as an international research group by merging of The Leeds Castle Polyposis Group (established in 1985) and The International Collaborative Group on HNPCC (established in 1991). The aim of the society is to encourage research into all aspects of gastrointestinal hereditary tumour syndromes, to educate physicians in molecular genetics and clinical management, to assist in establishing or maintaining a register and to provide a forum for presentation and discussion of scientific data as well as to facilitate collaborative studies. The society holds plenary scientific meeting every 2 years, and over the years a large number of collaborative studies have been carried out about FAP and HNPCC. The next meeting will be held in San Antonio, Texas in March 2011 [81].

35.10 Results of the Registries

Establishment of both polyposis and HNPCC-registers has improved the prognosis for families with hereditary risk of CRC and increased knowledge of the natural history of the syndromes by local research and many international collaboration

studies. Additional information to the public communities and the professional healthcare system has increased the awareness of hereditary cancer syndromes and the possibilities of genetic testing and surveillance.

A substantial reduction of the incidence of CRC from about 67% in symptomatic FAP patients to only few percent in asymptomatic screen-detected patients has been demonstrated [6, 69, 70, 82–84], and the most frequent cause of death has changed from primarily CRC towards duodenal cancer and desmoid tumour [85–88]. Major improvements in the survival have been demonstrated after the establishment of the polyposis registers [70, 89], and the FAP proportion of all colorectal cancers is almost halved from 0.12% before to 0.07% after establishment [69].

In a similar manner, HNPCC registers have contributed to the reduction in risk of CRC and CRC-related mortality [74–80, 90–97]. Surveillance results from the Finnish HNPCC register have led to a 63% reduction of CRC [72, 73], and several studies have shown that CRC is detected at an earlier stage compared to the stage in historical controls [72–75, 91–95].

In conclusion, the contribution of registers to the general control of colorectal cancer may be modest, but the impact on survival in FAP and HNPCC families is substantial, and registration does in fact save many lives.

35.11 Future Challenges: Electronic Data Exchange

The amount of genomic information has exploded during the last 10 years and many new genes have been identified with association to different diseases. In the future, we have to consider not only with monogenic but also polygenetic inheritance in families with hereditary CRC and presumably have to include information on modifier genes, when risk estimation is related to individual and familial phenotype.

Collection and handling of this large amount of data can only be dealt with by integrating biomedical and clinical information. Often family members are living in different parts of the country or even in different countries, which underline the necessity of being able to exchange information between different professionals taking part in the treatment of families with genetic diseases. Centralization of data in a register, with the access to information on relevant data for health professionals dealing with the individual patient, has the advantage of prohibiting double examinations and delayed diagnoses, and ensures scientific knowledge to establish the most optimal treatment. The data is needed to gain further information on disease and genomics in order to support genomic-based medicine. This collection of data does not only have a great advantage in the treatment of the patients and their family but also is crucial as a research tool.

In the majority of registers, data are submitted to the register in paper versions and typed into the database and corresponding pedigree program on location. Using modern information technology in form of electronically exchange of data is an obvious solution to overcome the workload dealing with enormous quantity of data

and ensure that all patients receive equal access to information. In developing tools adequate to deal with this problematic, we have to address the following questions or challenges:

- How can and should these data be collected and exchanged?
- What are the needs for interoperability of data and standards?
- What are the demands regarding data security, ethics and confidentiality?
- Which tools are available and required for data analysis and information retrieval from complex and heterogeneous databases?
- How can we deal with the fact that genetic information include data on the entire family and not only single individuals?

One of the collaborative studies in the InSiGHT community – INFOBIOMED has used the national Danish HNPCC register as a model for electronic data exchange between different departments taking care of diagnosis and screening (Further information: http://www.infobiomed.org). The most pronounced gaps identified in the project were the heterogeneous way of storage of data in the different departments and registers and the lack of standards for both clinical contents and terminology in the field of oncogenetic diseases. Another experience gained from the project was that development and implementation of system for electronic data exchange is very time-consuming and success in the process is interest and feedback from each of the parties participating.

One of the future challenges for the InSiGHT community is to define and agree upon the golden standard for hereditary colorectal cancer registers in order to facilitate exchange of data for coming research.

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Index

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