INHERITANCE OF KIDNEY AND URINARY TRACT DISEASES

TOPICS IN RENAL MEDICINE

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INHERITANCE OF KIDNEY AND URINARY TRACT DISEASES

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PREFACE

Genetic disorders have emerged as a prominent cause of morbidity and mortality among infants and adults. As many as 10% to 20% of hospital admissions and at least 10% of the mortality in this age group are due to inherited diseases.

There are at least two factors that have brought genetic disorders into the forefront of pediatrics. One is a great reduction in childhood mortality due to infections and nutritional deficiency states, and the other is the rapid progress made in the identification of genetic defects. Amniocentesis, chorionic villus sampling, and recombinant DNA technology have already had a tremendous impact on the practice of medicine. This is why the first two chapters of this volume are dedicated to general principles of molecular genetics and to a description of the techniques used to diagnose genetic disorders at the DNA level. The relevance of this new area of science to the study of inherited renal diseases is reflected in the large body of knowledge that has been generated regarding the association between various glomerular nephritides and genetic markers such as the HLA system, and even more impressively in the direct or indirect identification of abnormal genes or gene products in Alport's syndrome, autosomal dominant polycystic kidney disease, and Lowe's syndrome. These discoveries figure prominently in the pages of this book.

Yet, the progress we have made has barely scratched the surface of the problem. There remain a large number of diseases in which the mode of transmission is unclear and the genetic defect unknown. This is particularly

true of many types of malformation of the kidneys and urinary tract. Undoubtedly, during the years to come the number of conditions in this category will decrease. First the gene defects will be identified in monogenic hereditary disorders, then the molecular lesions associated with chromosomal abnormalities will be determined, and eventually the methods of genetic diagnosis will be extended to multifactorial diseases. It is therefore likely that in the not too distant future, many of the concepts expounded in this volume will have to be updated. We trust that all those who have worked hard to bring this publication to print will welcome such an opportunity. For we shall look forward to the time when for each disorder described in this volume the mutant gene and its chromosomal map location will be identified. This will truly open the gate to the golden age of medicine, when correction of gene abnormalities will become possible.

Adrian Spitzer
Ellis D. Avner

I. GENERAL PRINCIPLES

1. MOLECULAR BIOLOGY, GENE EXPRESSION, AND MEDICINE

JAMES P. CALVET

The discipline of molecular biology probably got its start sometime in the 1930s.[1] This was followed by the discovery in the 1940s that DNA is the genetic material, and by the elucidation of its structure in the 1950s. Since that time, there has been a revolution in molecular biology, and as a result, an explosion in our knowledge about basic cell function. A large number of molecular biology techniques have been standardized [1, 2] and even automated [3]; and many of these procedures are rapidly entering diagnostic laboratories.

This chapter will provide a conceptual and practical introduction to the field of molecular biology. It will review our present state of knowledge of gene structure and expression, and will outline some of the recombinant DNA procedures being used to investigate eukaryotic genomes. An attempt has been made to capture the elements of only the more important techniques as they are currently being put to use in a number of research settings. Additional information, both general and specific, may be obtained from a number of excellent books [4–8] and reviews [9–11].

GENES, mRNA, AND PROTEIN

The human genome has 23 pairs of chromosomes, one member of each pair being maternal and the other being paternal. Each chromosome is a linear molecule of double-stranded DNA consisting of the nucleotides adenosine (A), thymidine (T), guanosine (G), and cytosine (C). The two strands of DNA are complementary to each other, based on the principle that A can pair with

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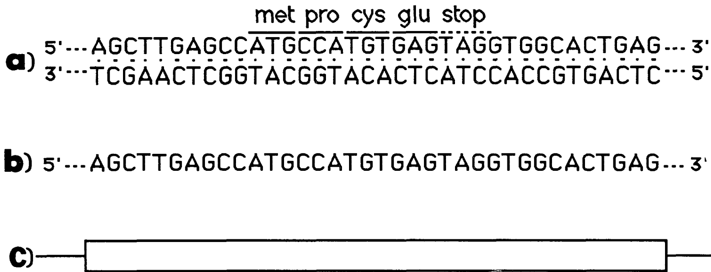

Figure 1-1. A DNA sequence. DNA is shown as an antiparallel base-paired molecule (**a**), as an abbreviated, single-stranded structure (**b**), and in its simplest, line and box form (**c**). The double-stranded version illustrates A–T and G–C base-pairing between the coding and noncoding strands. A short amino acid coding region is shown, with the noncoding strand above the coding strand. The methionine (ATG) initiation codon is follow by codons for proline, cysteine, and glutamic acid, and the sequence is terminated by a TAG stop codon. When DNA is depicted as a single strand, it is always the noncoding (mRNA-like) strand that is presented. In its simplest form, a gene is often represented as a box on a line.

T, and G can pair with C (figure 1-1). Thus, the chromosome is a sequence of complementary nucleotides or base-pairs; when DNA is sequenced, it is the order of these nucleotides that is determined. Each of the DNA strands in the double helix is a chain of nucleotides linked together by phosphodiester bonds that join the sugar moieties of adjacent nucleotides. Because these attach to the so-called 5′ and 3′ carbons of these sugars, there is a polarity to the chain that gives it a 5′ end and a 3′ end. The complementary DNA strands lie along one another in an antiparallel fashion, such that the nucleotide at the 5′ end of one strand is base-paired to the nucleotide at the 3′ end of the opposite strand. By convention, double-stranded DNA molecules are often reduced to single lines of nucleotides (representing only one of the two complementary strands), and genes are often depicted as rectangular boxes with the 5′ end on the left and the 3′ end on the right (figure 1-1).

Genes are functional units that encode information for the synthesis of protein (or for RNA molecules such as ribosomal RNA or transfer RNA as the end-products). They are discrete chromosomal locations lying along a DNA chain that is essentially featureless except for the nucleotide sequence itself. Genes are separated from one another by noninformational stretches of nucleotides (these will be faithfully sequenced along with the genes themselves when the human genome project is undertaken and completed). Each gene has at its side the regulatory elements that allow it to communicate with other genes via *trans*-acting factors that jump onto and scan along the DNA strand to find the gene units they are designed to regulate.

Some genes, such as the histone genes [12] or the globin genes [13], are known to exist in clusters, possibly for their coordinate regulation. However, most genes appear to be scattered around the genome without regard to their

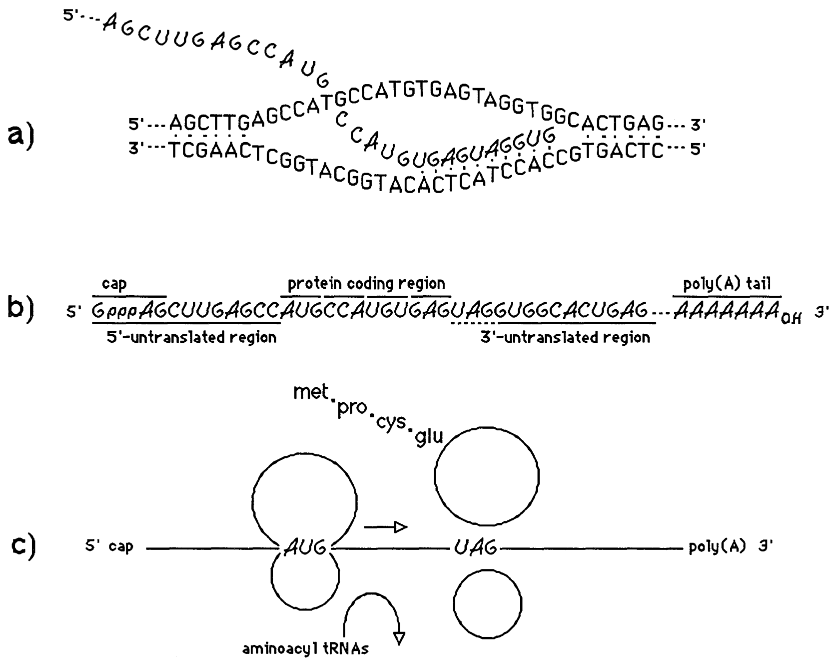

Figure 1-2. Transcription and translation. The process of gene transcription (**a**) gives rise to mRNA (**b**) which is then translated into protein (**c**). The DNA coding-strand specifies the mRNA sequence by the conventional base-pairing rules, except that uridine (U) is used in RNA instead of thymidine. mRNA has a cap at the 5′ end and a poly(A) tail at the 3′ end, both of which are added posttranscriptionally. The cap contains a G residue that is covalently attached to the mRNA by a 5′ to 5′ triphosphate bond. The protein coding region, which is flanked by 5′- and 3′-untranslated regions, begins with the AUG initiation codon and terminates with the UAG stop codon. Those triplets represent an open reading frame (ORF). Protein synthesis is initiated at the AUG by binding of the small and large ribosomal subunits to the initator region of the mRNA. The ribosome, together with the aminoacyl tRNAs, translates the mRNA by reading each codon and specifying the covalent addition of the proper amino acid to the growing polypeptide chain. Protein synthesis is terminated at the UAG.

function. Even the globin genes, while grouped as clusters of α-like and β-like coding sequences, lie on two different chromosomes. In fact, the chromosomal location of any particular gene was probably determined by evolutionary processes independent of function. Although chromosomal position effects are likely to have a role in gene expression, a gene, in principle, should be able to exist almost anywhere on any chromosome. Fortunately, therefore, once a gene is localized and isolated, most (if not all) of its regulatory regions can be expected to be in its proximity, and thus accessible to analysis. A number of genes, in fact, have been dissected from the chromosome (together with their regulatory regions) and have been shown to be functional when placed in a foreign environment [14] or back into a different chromosomal site [15].

Gene transcription gives rise to messenger RNA (mRNA) molecules that represent complementary copies of one of the two DNA strands of the double helix. The coding strand is copied into RNA according to the same rules governing DNA base-pairing, except that a uridine (U) is used in RNA instead of a thymidine (figure 1-2). Since the coding strand is complementary to mRNA, it is the noncoding strand that resembles the mRNA sequence. By convention, when a DNA sequence is shown in a figure as a single strand, it is always the noncoding strand (or the one that looks like the mRNA) that is presented (compare figures 1-1 and 1-2).

Messenger RNA has a protein-coding region within it that always begins with the initiating AUG methionine codon and ends with one of three termination codons, UAA, UAG, or UGA. The sequence context immediately flanking the AUG is thought to be important for determining the efficiency of initiation of protein synthesis [16]. Upstream of the AUG is a 5′ untranslated region and downstream of the termination codon is a 3′ untranslated region, which are thought to be involved in the regulation of protein synthesis. Sequences that govern mRNA turnover, for example, have been found to reside at the 3′ ends of certain mRNAs [17]. The very 5′ end of the mRNA has a methylated guanosine residue, termed a cap, which is added posttranscriptionally. The very 3′ end has a stretch of approximately 150–200 adenosine residues, also added posttranscriptionally, which composes the poly(A) tail [18]. Virtually all mRNAs are 5′ capped and 3′ polyadenylated, and because of their additional 5′ and 3′ untranslated sequences can be considerably longer than would be expected from the sizes of the proteins they encode.

All of the 64 possible combinations of three nucleotide (triplet) codons containing any of the four bases at each position ($4 \times 4 \times 4 = 64$) are utilized in protein synthesis. Each codon (with the exception of the three used for termination) specifies a unique amino acid. Since there are only 20 amino acids, it follows that some are specified by more than one codon. The AUG initiation codon determines the start site for protein synthesis and establishes the reading frame (figure 1-2). From that point on, every codon is read (or translated) sequentially in a 5′ to 3′ direction, directing the tRNAs to incorporate the appropriate amino acids into the growing polypeptide chain [19]. A nucleotide sequence flanked by an AUG codon at one end and a termination codon at the other end is called an open reading frame (ORF) because it has the potential to be translated into protein. In practice, open reading frames are important to look for when sequencing DNA, since gene regions encoding proteins can often be recognized this way. In fact, the power of molecular biology is perhaps best illustrated by our ability to find and sequence genes, and then to translate them on paper to predict the amino acid sequences of their protein end-products without knowing ahead of time very much (if anything) about the gene or the encoded protein.

Genetic evidence (and other means of guessing) have suggested that the human genome contains approximately 50,000 to 100,000 genes. The differ-

ential expression of these genes is responsible for the enormous diversity of cellular specialization that goes into producing an organism or individual. The regulation of cell- and tissue-specific gene expression is currently under active investigation, and as might be expected is turning out to be terribly complex. Cells are different from one another because of the proteins they make. These proteins are a reflection of gene activity itself, and of other processes that regulate the levels of individual species of mRNA. The amount of a particular protein being synthesized is often a function of the level of its mRNA, and thus it is frequently possible to determine what a cell is doing by looking at its population of mRNAs. Recombinant DNA techniques provide us with the ability to isolate individual genes so that they can be analyzed to determine how they are regulated and what, specifically, they code for. In fact, it is now possible, by using recombinant DNA techniques, to identify differentially expressed genes (without knowing ahead of time what one is looking for) by identifying differences in the levels of mRNAs between different cell types, or in the same cell under different conditions.

RECOMBINANT DNA

As its name implies, recombinant DNA involves the joining of DNA molecules from different sources to create novel combinations of genetic material. The purpose of doing this is to facilitate the handling of large, complex genomes by breaking them down into much smaller and more manageable fragments. This technology has revolutionized the study of even the smallest viral and bacterial genomes, and has been exceedingly useful for studying the larger genomes of higher organisms. The 50,000 to 100,000 genes in the human genome lie in approximately three billion base-pairs of DNA. If a gene is 3000 base-pairs in length, it represents one part in a million. In reality, it is impossible to purify genes using conventional biochemical techniques (such as columns and gradients, commonly used to purify proteins). This is because, biochemically, the properties of one gene resemble those of any another; although there may be slight differences in base composition from one gene to another, these differences are not sufficient to provide a useful biochemical handle. What really distinguishes one gene from another is its sequence, and it is this sequence information that provides the recombinant DNA handle.

Restriction enzymes

Many of the tools of recombinant DNA research can be extremely precise. Among the most useful of these tools are the restriction enzymes—so useful, in fact, that their discovery gave Werner Arber, Hamilton Smith, and Daniel Nathans the Nobel Prize in 1978 [20–22]. The unique property of these enzymes is that they can cleave DNA in a sequence-specific fashion, and thus will reproducibly generate a specific and characteristic set of fragments from any source of DNA. These enzymes provide the most basic instrument for isolating a gene, because they can utilize nucleotide-sequence information to

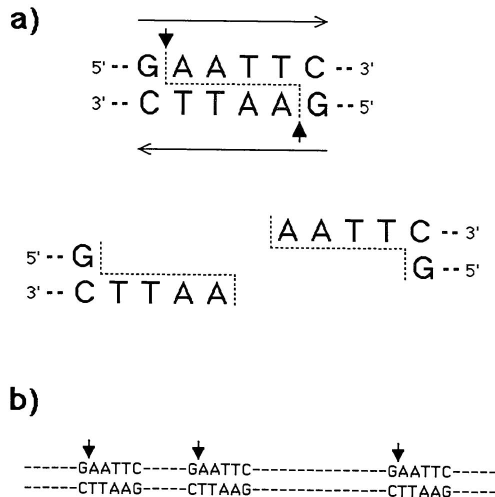

Figure 1-3. EcoRI restriction endonuclease cleavage site. The six base-pair recognition sequence for EcoRI (**a**) has two-fold rotational symmetry (leftward and rightward arrows). The restriction enzyme cleaves between the G and A on each strand, giving rise to cut ends having complementary sequences (below). Restriction mapping (**b**) provides a means to locate cleavage sites for particular restriction enzymes within a larger DNA molecule. Restriction fragments produced by enzymatic digestion can be isolated and recombined with other similarly cut DNAs by annealing their cohesive complementary ends.

cut out and remove a gene from its chromosomal site. Restriction enzymes are essential for recombinant DNA purposes because they generate specific DNA fragments that can be joined with other DNA fragments cut with the same enzyme (or enzymes that produce compatible ends) to create novel DNA sequence combinations.

More than 800 restriction enzymes, having over 100 different specificities, have now been purified, and many of these are commercially available [23, 24]. These enzymes are isolated from bacteria, where they are used for the protection of the bacterial cell from invasion by foreign DNA. By convention, restriction enzymes are named after the genus and species of the bacteria from which they were isolated. (For example, EcoRI is isolated from *E. coli* and PstI is isolated from *P. stuartii.*) Restriction enzymes are one of the components of a dual restriction–modification system [25]. The bacterial cell produces enzymes that modify its own DNA by the methylation of certain nucleotides at DNA sites that are also specifically recognized (and cleaved) by its own restriction enzymes. Methylation of these sites prevents them from being cleaved. Thus, the bacterial cell protects its own DNA from its own restriction enzymes, while invading (e.g., viral) DNA that is not methylated is recognized as foreign and is inactivated by being cut up into fragments.

Most restriction enzymes used for recombinant DNA purposes recognize

specific 4-base, 5-base, or 6-base symmetrical DNA sequences (figure 1-3). These DNA sites have two-fold symmetry because the same nucleotide sequences, read with 5′ to 3′ polarity, exist base-paired with one another on the opposite, complementary strands. The enzyme sees the same sequence on each strand and cleaves between the same two nucleotides within that sequence. The cleavage sites on the two DNA strands may be directly opposite one another, giving rise to blunt-ended double-stranded molecules, or may be staggered with respect to one another, giving rise to overhanging or protruding ends of 2–4 nucleotides at the cut sites. While both types of ends can be used in forming recombinant molecules, the overhanging complementary ends produced by staggered cuts are particularly useful because they allow different DNA molecules having identical ends to anneal with each other so that they can be joined.

The frequency with which restriction sites can be found in DNA depends on the particular sequence. As an approximation, 4-, 5-, and 6-base cutters would find their sites on average every 256, 1024, and 4096 base-pairs in a random stretch of DNA. In practical terms, this means that a 4-base cutter may have ten or so sites in a gene that is approximately 3000 base-pairs in length, whereas a 6-base cutter may or may not have a site in that gene. The recognition sites for hundreds of restriction enzymes are known [24], and thus, using a computer program, it should be possible to predict where any of these enzymes will cleave a particular nucleotide sequence. If the sequence of a gene is not known, restriction sites can be mapped by cutting the DNA with various combinations of enzymes, then resolving the fragments by gel electrophoresis and piecing the puzzle back together (figure 1-3).

If the desired gene (or gene piece) is not flanked by restriction sites that are convenient for a particularly cloning protocol, the sites can actually be supplied artificially [26]. The recognition sequences are synthesized as short double-stranded oligonucleotides that can be added to the ends of the DNA fragment and then cut with the appropriate restriction enzyme, giving rise to overhanging cohesive ends that will facilitate the joining of other, similarly cut DNA molecules. Thus, one can make use of the convenience of restriction enzyme technology by furnishing the appropriate restriction sites in order to clone virtually any DNA molecule of interest.

Host/vector systems

Genes are purified by the process of molecular cloning [27]. This is achieved by getting the sequence of interest into a DNA vector and propagating the recombinant molecule in a bacterial host. There are two types of vectors, each suiting a different purpose. Plasmids are small, circular DNAs that can replicate autonomously in bacteria, and in essence behave as minichromosomes. Bacteriophage (or phage) are bacterial viruses that can carry recombinant DNA sequences, and propagate themselves as any virus would. Both kinds of cloning vectors can accept pieces of foreign DNA and still function in the host.

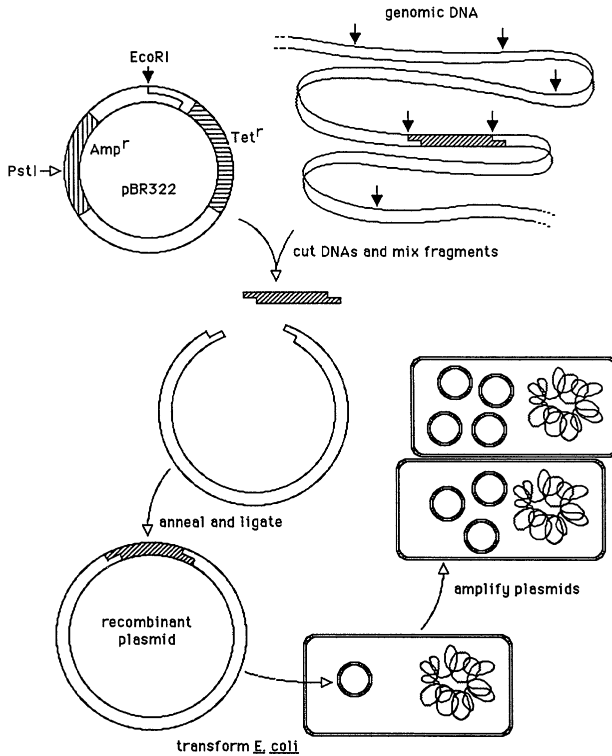

Figure 1-4. DNA cloning. The plasmid DNA cloning vector pBR322 has antibiotic resistance genes for ampicillin (Amp^r) and tetracycline (Tet^r), and contains several unique restriction sites, including one for EcoRI which is located between the two drug-resistance genes. Recombinant clones are constructed, in this example, by digesting pBR322 and genomic DNA with EcoRI. The fragments are mixed together, annealed and ligated, giving rise to a population of recombinant plasmids that are then used to tranform *E. coli*. The plasmids are represented as small circles compared to the much larger bacterial chromosome, which is represented as a tangled line. The plasmid begins to replicate once it enters the bacterial cell, and together with the replicating bacteria results in a colony that contains many copies of a single recombinant molecule. There is also a single PstI restriction site in the ampicillin-resistance gene. To clone a foreign gene into this site, the plasmid would be cut with PstI and the insert would be ligated into this site. The construct would then be used to transform *E. coli*, and tetracycline-resistant bacteria would be selected. By also selecting for cells that are ampicillin-sensitive on a duplicate plate, transformants would be identified that contain a piece of foreign DNA inserted into the ampicillin-resistance gene, thus inactivating it.

In both cases, the vector DNA is cut with an appropriate restriction enzyme (figure 1-4), the foreign DNA (having ends compatible with those of the vector) is annealed with the vector DNA, and a recombinant molecule is constructed by ligating the ends of these DNA molecules together with the enzyme, DNA ligase. These recombinant molecules are then introduced into

a host, where they replicate into a large number of identical copies of the original recombinant molecule.

Recombinant plasmids are introduced into bacteria by transformation. Under certain conditions bacteria will take up DNA molecules, especially if the DNA is a closed circle (thus favoring recombinant molecules over those that were cut, but not ligated to the foreign DNA). In practice, a culture of (restriction-enzyme-deficient) bacteria is made ready and mixed with the recombinant plasmids. DNA is taken up by perhaps one in one million cells, and begins to replicate by making use of the DNA synthetic enzymes of the host. These cells are easily identified because plasmids are constructed to contain antibiotic-resistance genes, which are expressed when the plasmid takes up residence in the host. The bacteria are plated on antibiotic-containing agar plates, and the so-called transformants are grown up as isolated colonies.

A bacterial cell is transformed by one and only one recombinant plasmid (because the process is an infrequent event statistically). Thus, all of the replicated copies of that original plasmid in the initial transformant and all of the plasmid copies in all the cells in the bacterial colony derived from that initial, transformed bacterial cell are identical. Once a colony has grown up, it is then a relatively simple matter to transfer it to broth and to grow up virtually unlimited quantities of a pure recombinant plasmid.

Recombinant bacteriophage are propagated by a somewhat different procedure. After construction of the chimeric DNA molecule, a functional virus particle is assembled in vitro by incubating the DNA with a packaging mixture containing all the necessary viral proteins. The packaged phage are then used to infect a culture of bacteria. If the ratio of virus to bacteria is kept low, as with transformation, most cells will not get infected, and those that do will be infected with only one virus particle. These are then plated out on a lawn of bacteria on agar and allowed to grow. Infected cells are few and far between. As the virus replicates and lyses its original host cell, its progeny invade surrounding cells which, in turn, lyse and spread the infection outward from the initial, infected cell. This creates a small clear area, or plaque, on the culture plate, which contains the countless identical progeny of the original recombinent. Bacteriophage from this plaque can then be isolated and used to start as many large-scale preparations as necessary.

Cloning vectors can be engineered in any number of ways to suit almost any purpose [1]. Armed with a battery of restriction enzymes and other tools to manipulate DNA, as well as with nucleotide sequence information about the vector and an understanding of the biology of prokaryotic and eukaryotic gene expression, one can design and execute almost anything imaginable. An example of a popular and well-designed plasmid cloning vector is pBR322 (figure 1-4). It is approximately 4.3 kilobases (Kb) in size, has a number of unique restriction sites, contains an origin of DNA replication, and has two drug-resistance genes (for ampicillin and tetracycline). In general, DNA molecules of greater than approximately 10–15 Kb are not handled well in pBR322.

Since many eukaryotic genes are considerably larger than this size, plasmids can have their limitations, and for cloning larger DNA molecules it may be preferable to use a phage vector. Lambda bacteriophage is frequently used for these purposes. Consequently, a number of lambda derivatives have been engineered to meet various cloning needs. The lambda Charon vectors [28], for example, have been used frequently for the construction of genomic libraries. This virus has a genome size of approximately 49 Kb and a region (containing genes that are dispensible for vegetative growth of the virus) that can be removed and replaced with foreign DNA of approximately 15–20 Kb in size. It is possible to clone even larger pieces of DNA by using a hybrid phage/plasmid vector called a cosmid [29, 30], which combines some of the more useful features of the two. This construct has the origin of replication and selectable drug-resistance genes of the plasmid, and the packaging (or cos) sequences of the bacteriophage. This relatively small plasmid, which can accept DNA molecules of up to 35–45 Kb, can be packaged into a pseudo-virus particle and introduced into cells by transduction (infection), rather than transformation.

A number of plasmids and phages have been designed to express foreign DNA inserts as RNA transcripts and as protein products. If an RNA transcript of a particular DNA sequence is desired, for example, to use as a radioactively labeled hybridization probe or as a synthetic mRNA to be translated into protein, the DNA sequence can be cloned into one of the SP6 transcription vectors. These are derivatives of pBR322 that contain promoters for bacteriophage SP6 polymerase adjacent to a short, so-called multiple cloning region that has a number of convenient restriction sites for placing DNA inserts. Once cloned, the purified recombinant plasmid can be transcribed in vitro with purified bacteriophage RNA polymerase to produce relatively short (approximately 100–300 nucleotides), high-specific-activity radioactive probes [31], or relatively long (several Kb) unlabeled mRNA products [32]. For additional convenience, there are more versatile vectors. These have a multiple cloning region flanked by an SP6 promoter on one side and a T7 promoter on the other side, or alternatively, a multiple cloning region flanked by T7 and T3 promoters. By chosing two different restriction sites within the multiple cloning region that correspond to the different, cut ends of a DNA fragment to be cloned, one can insert the DNA into the vector with a predicted orientation. Transcription of the fragment with one of the two RNA polymerases will give rise to a sense-strand RNA product (e.g., an mRNA). Transcription with the other RNA polymerase will give rise to an antisense product (e.g., a hybridization probe to detect the complementary mRNA).

Expression of cloned genes as proteins in bacteria and in eukaryotic cells has been used to select transformants and to screen libraries, to analyze the in vivo functions of genes, and to produce large amounts of recombinant proteins. Plasmid and phage vectors (e.g., pUC and lambda gt11) that contain the *E. coli* β-galactosidase gene have been developed to facilitate selection and screening

[33, 34]. The β-galactosidase gene has a multiple cloning site placed within it (in an inocuous position) for cloning a foreign piece of DNA. Proper functioning of this gene in *E. coli* can be monitored by reaction of a dye that indicates the presence of β-galactosidase enzyme. However, insertion of foreign DNA into the β-galactosidase gene interrupts the coding sequence and prevents its expression. Thus, plasmid colonies or phage plaques that contain recombinants appear white or clear, in contrast to those that lack recombinants, which appear blue.

Eukaryotic expression vectors for analysis of gene regulation and protein function make use of plasmid constructs and viruses that provide an efficient means of introducing genes into cells and of expressing them once they are there [35, 36]. A typical vector may have plasmid sequences that include a bacterial origin of DNA replication and a selectable drug-resistance marker so that the eukaryotic gene can be cloned and propagated in *E. coli*. It will also have a eukaryotic origin of replication, such as the SV40 viral origin (so that it will replicate in certain eukaryotic cells), and additional sequences that will increase the efficiency of transcription and mRNA production [37]. These vectors can be introduced into cultured cells by DNA-mediated transfection [38, 39], a technique that involves the coprecipitation of DNA with calcium phosphate or DEAE-dextran, which facilitate the entry of DNA into cells. For higher-efficiency transfection, cells can be treated with short, high-voltage electrical pulses that produce small pores in the plasma membrane (electroporation) through which the DNA enters the cell [40]. Once taken up, the DNA is actively but transiently expressed, and can be assayed in a matter of 2–3 days. Cotransfection of the vector with a marker that is selectable in a eukaryotic cell can facilitate the establishment of stable transformants. If these cells are grown in culture for longer periods of time, some of the cells will become permanently transformed by the relatively inefficient integration of the transfected DNA into the chromosomal DNA of the host genome. These stably transformed cells are useful for long-term studies of gene expression. Viral expression vectors, such as bovine papillomavirus (BPV) or certain murine retroviruses, have additional advantages. BPV [41–43] can reach high copy numbers and be maintained for long periods as episomes (unintegrated). Murine retroviruses can be introduced into cells with high efficiency and can be stably integrated into genomes [44].

The production of recombinant proteins in bacteria [45] requires that a gene sequence be cloned adjacent to a strong promoter so that high levels of mRNA can be obtained. It is also advantageous to make use of a promoter that can be regulated in the cell so that the production of the protein can be delayed until the bacterial culture has reached its optimum density. This latter point is important, since eukaryotic proteins are often toxic to bacterial cells, especially if they are synthesized in large amounts. Proteins produced in bacteria in high concentrations often form inclusion bodies and are sometimes difficult to get into solution for purification. It should also be recognized that posttrans-

lational modifications, such as proteolytic processing, phosphorylation, and glycosylation, will not occur with eukaryotic proteins synthesized in bacteria, thus compromising their biological activity. Some of these problems can be circumvented by expressing recombinant proteins in eukaryotic cells. For example, the baculovirus expression system [46], in which recombinant viruses are grown in cultured insect cells or in insect larvae, have proven to be particularly useful for the high-level expression (synthesis, modification, targeting, and/or secretion) of many eukaryotic proteins, in part because of their very strong polyhedrin and p10 promoters, and also because these promoters are active very late in the virus life cycle, allowing production of the recombinant proteins at a time that does not interfere with viral replication.

Library construction and screening

Libraries are resources of genetic information. They consist of collections of clones that are constructed using recombinant DNA techniques, and they are screened using molecular probes to retrieve the clones of interest. There are two kinds of libraries: genomic libraries [47], which contain fragments of chromosomal DNA (the genes themselves), and cDNA libraries [48], which contain DNA copies of mRNAs (the expressed gene sequences). Lambda bacteriophage is used frequently for both genomic and cDNA libraries. Cosmids may be used for cloning larger genomic DNA fragments, and plasmids may be used for libraries made up of the generally smaller cDNAs.

Genomic libraries can be used 1) to isolate a gene in order to obtain information about its structure, sequence, or the location and sequence of its regulatory regions; 2) to investigate the molecular basis of a genetic disease (in this case, a genomic library is constructed from the DNA of an individual with an inherited disease, or from a particular cell type that is known to contain a mutated or abnormally functioning gene; and 3) as a means to locate and identify the gene responsible for a particular disease, for which nothing else except the phenotype is known.

In contrast to genomic clones, cDNA clones represent copies of the expressed genes in a particular cell, tissue, or organ. Therefore, 1) cDNA libraries can be used as a means to identify and isolate cell-specific expressed sequences; 2) cDNA clones can be sequenced to determine the primary amino acid structure of their encoded proteins; and 3) cDNAs can be expressed as protein products in bacteria, and thus can be used to produce recombinant eukaryotic proteins in prokaryotic hosts.

Genomic libraries

DNA for constructing genomic libraries can be isolated from any cell, tissue, or organ that is convenient to handle. A human library, for example, can be made from the DNA in the circulating lymphocytes from as little as 10–15 ml of blood. High-molecular-weight DNA is extracted and purified, digested (partially) with an appropriate restriction enzyme to generate a range of

different size fragments, and size-fractionated by sucrose gradient centrifugation or gel electrophoresis. This DNA is then mixed with lambda DNA that has been digested with a compatible restriction enzyme, the fragments are enzymatically ligated together, and the recombinants are packaged into virions using an in vitro packaging mixture [49]. A culture of *E. coli* is then infected with the recombinant bacteriophage, and the number of infectious units is determined by titrating the library. The number of plaque-forming units indicates how many recombinant viruses were initially made and therefore how representative the library is [50]. The library is then amplified by carrying out a large-scale infection. The recombinant phage resulting from this process can be stored almost indefinitely and used whenever necessary to grow new preparations for screening.

Genomic libraries should contain enough clones to include all of the DNA sequences in the genome [51, 52]. Genomic DNA is randomly cleaved with restriction enzymes to generate a variety of overlapping fragments of different sizes. Optimally sized fragments are then packaged into enough phage particles so that (statistically) every sequence is represented in the library. Partial digestion with restriction enzymes that are 4-base cutters provides the best opportunity to obtain fragments that are both optimal in size and representative, given the fact that 4-base cutters have sites every 256 bp on average (in a random sequence) and that the optimal-size clone is more than 50 times this size. It is not necessary to cut out genes as discrete, self-contained units. In fact, many genes are much larger than the 15–20 Kb that can be packaged into lambda phage, and it is usually no problem to isolate a family of related (overlapping) clones that represent the entire gene region, no matter how large the gene [50].

The size of the human genome is approximately 3×10^9 bp. If it were possible to divide it up into nonrandomly generated fragments of 15–20 Kb in size, a complete human library would need only 150,000–200,000 of these ideal clones. In practice, a human library (constructed of randomly generated fragments) would actually require about 700,000–900,000 clones to ensure a 99% probability that any particular gene is represented at least once [53].

cDNA libraries

mRNA for constructing cDNA libraries can be purified from any cell, tissue, or organ of interest. Since mRNA usually comprises less than 5% of the total RNA of a cell (much of the rest being ribosomal RNA and transfer RNA), it is usually advantageous to enrich for poly(A)$^+$ mRNA by one or two rounds of oligo(dT)-cellulose chromatography [54]. This is carried out by applying the RNA to a column containing cellulose to which short (15–18 bases) oligo(dT) nucleotides are covalently attached. mRNA containing poly(A) tails (most mRNAs) will anneal to the oligo(dT) by A–T base-pairing in a high-salt buffer, allowing poly(A)$^-$ RNAs to pass through. The mRNA is then removed with a low-salt elution. The integrity of the purified mRNA can be evaluated

by gel electrophoresis, Northern hybridization, or in vitro translation to determine whether the preparation is sufficiently intact to yield successful results.

Double-stranded (ds) cDNA is generated from mRNA in two steps. The first cDNA strand is synthesized (using mRNA as a template) by reverse transcriptase, an enzyme that is purified from avian myeloblastosis virus or Moloney murine leukemia virus. This enzyme is normally found packaged in the retrovirus particle, and is used to replicate the single-stranded viral RNA genome into double-stranded DNA for integration into the host chromosome. The enzyme actually has two activities: an RNA-dependent DNA polymerase activity (used for first-strand synthesis) and a DNA-dependent DNA polymerase activity (which, in some procedures, has been used for second-strand synthesis).

Oligo(dT), to be used as a primer for first-strand synthesis, is hybridized to the poly(A) tail of the purified mRNA, and a cDNA strand is synthesized from this primer using reverse transcriptase [55]. Completion of the first strand results in an mRNA/cDNA hybrid (cDNA for complementary or copy DNA). A number of different procedures exist for second-strand synthesis. In one procedure that is now used frequently [56–58], the mRNA strand of this mRNA/cDNA hybrid is nicked at numerous sites by the enzyme RNase H (H for hybrid), which is specific for the RNA strand of an RNA/DNA hybrid. The resulting 3′ ends of these RNA fragments are then used as primers for second-strand synthesis, using DNA polymerase I. This enzyme synthesizes DNA from the 3′ end of the primer in a 5′ to 3′ direction (with respect to the new strand being synthesized). At the same time, a 5′ to 3′ exonuclease activity associated with the enzyme removes the RNA (or DNA) strand lying downstream in its path (see discussion of nick-translation, below). The double-stranded cDNA products are prepared for cloning by first making the ends compatible with those of the vector. Ragged ends are made blunt by enzymatic trimming or filling reactions, and synthetic restriction enzyme sites are ligated onto the blunt ends using the enzyme DNA ligase. Addition of these so-called linkers (for example, for EcoRI) makes it possible to anneal the cDNAs to a suitably cut cloning vector.

The lambda vectors λgt10 and λgt11 are designed for cDNA cloning [34, 59]. These vectors are used when screening is carried out with hybridization probes (λgt10) or with antibody probes (λgt11). Both of these vectors have unique EcoRI sites (for cDNAs with EcoRI linkers) and are capable of accepting inserts of up to 7 Kb, a size sufficient to handle almost any mRNA (as a cDNA). As an alternative strategy, double-stranded cDNAs can be given homopolymer tails and then cloned into plasmid vectors. After cDNA synthesis, oligo(dC) tails are added to the 3′ ends of the double-stranded molecules using the enzyme, terminal deoxynucleotidyl transferase. The plasmid cloning vector is cut at a unique site (for example, the PstI site in pBR322) and is tailed with the complementary oligonucleotide, in this case

oligo(dG). The two are then mixed and annealed, and library construction is completed by transforming *E. coli* with the population of recombinants. If the PstI site is used, it is regenerated in the recombinant clones so that cDNA inserts can be released by digesting with PstI.

Successful library construction depends on the quality and the quantity of clones [60]. Since cDNAs are made by priming with oligo(dT), cDNA synthesis begins at or near the poly(A) tail at the 3′ end of the mRNA and proceeds toward the 5′ end. If any mRNAs are partially degraded (for example, cut in two), cDNA synthesis will go up to that point and stop. It is also possible (depending on a number of factors) for reverse transcriptase to stop synthesis prematurely. Thus, in a population of cDNA clones the 3′ ends may be overrepresented and the 5′ ends may be underrepresented. If full-length cDNA clones are necessary (they are not for some applications), it is important to start with intact mRNA, and to utilize procedures that will maximize full-length cDNA synthesis.

The number of clones required for success depends on the purpose of constructing the library [34, 48]. For example, if one wants to isolate a clone for an mRNA that makes up 10% of the mRNA population of a cell, a library containing only a few hundred clones would be perfectly adequate. On the other hand, if one wants to isolate a clone for a rare mRNA, or if one wants a more representative library, many more clones would be necessary. Of the several-hundred-thousand mRNAs in a typical cell, the vast majority are relatively rare to moderately abundant and are present at about 10 to 100 copies per cell. cDNA libraries for these mRNAs would have to contain 10,000 to 100,000 clones to be fully representative. Libraries for the rarest mRNAs would require up to a million clones. In planning a library, therefore, it would be advantageous to use a cell that is known to express high levels of the mRNA of interest, or to specifically induce the cell to produce high levels of that mRNA. Alternatively, it may be possible to enrich the mRNA preparation by size selection, hybridization to a related clone (for example, from another species), or by immune precipitation of the polysomes containing that mRNA.

Screening

After constructing a library (genomic or cDNA), it is next required that the clone of interest be identified and recovered [61]. In practice, the two most common ways to screen a library are by hybridization (with a nucleic acid probe) and by immunological methods (with an antibody probe). Of the two, hybridization is the more general approach [62], since immunological techniques can only be used when screening cDNA expression libraries.

HYBRIDIZATION. One of the cornerstones of molecular biology is the ability of complementary DNA or RNA strands to recognize each other and base-pair in a highly specific manner [63]. Double-stranded DNA consists of perfectly complementary, base-paired strands. If these are separated (denatured or

melted) into their constituent single strands by high pH or high temperature and then incubated under conditions that favor the re-formation of duplexes, there will be a trial-and-error process of DNA strands interacting with each other and finding complementary sequences. If these interacting DNA strands are sufficiently complementary to form stably base-paired duplexes (under the incubation conditions), they will remain associated with each other. Such duplexes are said to be renatured or reassociated.

RNA strands can also base-pair with single-stranded DNA (or with other RNA strands); if this occurs, they are said to be hybridized. Generally speaking, the term hybridization applies to the formation of any duplex of nucleic acid strands (DNA/DNA, DNA/RNA, or RNA/RNA) that was not originally base-paired. For example, the detection of a particular gene by Southern blotting may be carried out by the hybridization of a labeled recombinant DNA probe to restriction-cut genomic DNA. By the same token, the detection of a genomic or cDNA clone in a phage library may be carried out by the hybridization of a labeled probe to the DNA in the recombinant phage plaques comprising the library.

Hybridization conditions are important in the design of an experiment. The factors that influence the formation and stability of hybrids, and that have to be considered in setting up a hybridization reaction, are incubation temperature and salt concentration. In general, the optimum temperature is approximately 25°C below the temperature required to denature the same hybrids. If the reaction is carried out in the an aqueous buffer, the hybridization temperature is usually around 60–65°C. If the reaction is carried out in a buffer containing formamide (which lowers the melting temperature), the optimum may be around 40–45°C. Increasing salt concentration (usually NaCl) increases the stability of hybrids. Since the melting temperature and the rate of hybridization are both affected by salt concentration, both salt and temperature can be adjusted to suit the needs of the experiment.

Together, the conditions of salt and temperature are termed the stringency of the reaction. Increased stringency is achieved by increasing the hybridization temperature and decreasing the salt concentration; decreased stringency is obtained by decreasing the temperature and increasing the salt. For perfectly matched DNA or RNA strands, there is a set of standard criteria of salt and temperature that provides an optimal rate of hybridization. In practice, however, there is almost always the potential for illegitimate cross-hybridization because of the large number of similar (but not identical) sequences in the genome, and in mRNA populations. If cross-hybridization is a problem, the stringency can be increased to reduce nonspecific interactions.

Some experimental strategies require a certain amount of cross-hybridization. If, for example, one wants to isolate a gene for a human kidney-specific protein using a rat cDNA clone, conditions would have to be found that would allow cross-hybridization between the evolutionarily divergent sequences. This can usually be accomplished by decreasing the stringency. In

these cases, one must often strike a balance (usually arrived at empirically) that will tolerate a degree of relatively specific cross-hybridization, but will discourage unwanted, nonspecific interactions.

PROBES. Hybridization probes are usually labeled with ^{32}P by the process of nick-translation [64, 65]. This is a technique by which probe-specific DNA is actually synthesized in vitro in the presence of all four (one labeled with a ^{32}P atom) deoxynucleoside triphosphates (dNTPs). As these are incorporated into the newly synthesized DNA chain, the ^{32}P becomes a part of the phosphodiester backbone. The procedure makes use of a DNA clone for a specific gene (or gene segment) or cDNA. First, single-stranded breaks (or nicks) are generated at random spots in the double-stranded DNA molecule. These nicks are spaced sufficiently far apart so that no two (on opposite strands) are close enough to produce a double-stranded break. Second, DNA polymerase is used to repair the nicks (in the presence of the labeled dNTPs). However, in doing so, the DNA polymerase (using an endogenous 5′ to 3′ exonuclease activity) nibbles along the DNA ahead of it while simultaneously filling in the gap that it leaves behind. In essence, the original nick gets moved (or translated) along the DNA strand. This synthetic process takes place at every nick on both DNA strands, making use of the existing, opposite strands as the templates for new DNA synthesis. As such, most of the original DNA is replaced with a faithful copy of newly synthesized, labeled DNA. Nick-translated hybridization probes can be radioactively labeled to high specific activity, limited only by the specific activity of the labeled dNTPs and the degree of replacement synthesis that occurs.

Several alternative ways exist to label DNA. One technique coming into general use is the random primer approach [66], which makes use of a collection of short (hexanucleotide) DNAs that represent all of the possible combinations of six nucleotide sequences. These are hybridized to the denatured (single-stranded) probe DNA and are used as primers for DNA synthesis, again in the presence of labeled dNTPs. The denatured probe molecules, hybridized with a limiting amount of oligonucleotide primers, provide an opportunity for all the DNA sequence information on both strands of the probe to be copied and labeled.

Synthetic oligonucleotides can be used directly as probes [67] if there is sequence information about the gene or cDNA being screened for. The oligonucleotides are chemically synthesized [68] in an automated DNA synthesizer (available in most research centers) and labeled prior to hybridization by the enzymatic addition of ^{32}P to their 5′ ends. Specific hybrids can usually be expected for oligonucleotides that are at least 15–20 nucleotides in length, although longer probes can be easily made. Hybridization probes can be synthesized, based on amino acid sequence information for a particular protein, that will detect the gene or cDNA clone specific to that protein. In these cases, it is usually best to find a region of the protein that has an amino acid sequence specified by a relatively unambiguous set of codons. While some

amino acids are specified by up to six different codons, others have only one or two codons. The oligonucleotide probe is synthesized as a mixture of DNAs comprising all possible nucleotide sequences that could generate that peptide. These are then labeled and hybridized to the library as a mixture, with the expectation that one of the labeled sequences will match the gene exactly, and will hybridize specifically. DNA probes (labeled by nick-translation or with random primers) are double-stranded at the completion of the labeling reaction and have to be denatured prior to hybridization. Because they are double-stranded, they have the potential to hybridize to both DNA strands of the molecules that are being probed, no matter whether a Southern blot or a library is being screened.

Single-stranded nucleic acid probes can also be used for most hybridization purposes. The most frequently employed procedure for making single-stranded probes is to utilize one of the transcription vectors to make a labeled, single-stranded RNA [31]. The DNA sequence that is used as the template for probe construction is cloned adjacent to an SP6, T3, or T7 bacteriophage promoter, and RNA is made in vitro in the presence of ^{32}P-labeled nucleoside triphophates (NTPs). These probes can be used for almost any hybridization purpose, and have the advantage that unhybridized, single-stranded probe can be removed by ribonuclease treatment to reduce background radioactivity.

PLAQUE HYBRIDIZATION/COLONY HYBRIDIZATION. Phage libraries and plasmid libraries are screened by plaque hybridization and colony hybridization, respectively [69]. A genomic library that has been cloned in one of the phage vectors, for example, can be screened by hybridizing a labeled probe to the bacteriophage plaques, which represent the different recombinant clones. A portion of the library (which may consist of hundreds of thousands of different recombinants) is plated out by mixing the purified phage with a vast excess of bacteria to initiate an infection. The infected (and uninfected) bacteria are then spread onto a number of agar plates. Plating is carried out at a dilution that will allow individual plaques (each representing a clone) to be resolved from one another as they appear on the otherwise uniform lawn of bacteria (figure 1-5). A replica of the phage plate is then made by transferring some of the bacteriophage from each plaque onto a nitrocellulose sheet or circular nitrocellulose filter [70]. This is accomplished by laying the filter onto the agar plate and allowing some of the phage to adsorb to the nitrocellulose. The phage are then lysed in place to release their DNA. During this process the DNA is denatured into single strands, which adhere to the nitrocellulose (again, in place). In effect, therefore, a replica is made that mirrors the pattern of the phage plaques on the original plate, with a small spot of DNA on the nitrocellulose filter corresponding to each clone.

The phage plaques are screened by hybridizing the nitrocellulose replicas with a labeled probe. The nitrocellulose is placed in hybridization solution containing the probe in a sealed plastic bag, shallow pan, or covered petri dish, and is incubated (usually overnight) at the appropriate temperature. After

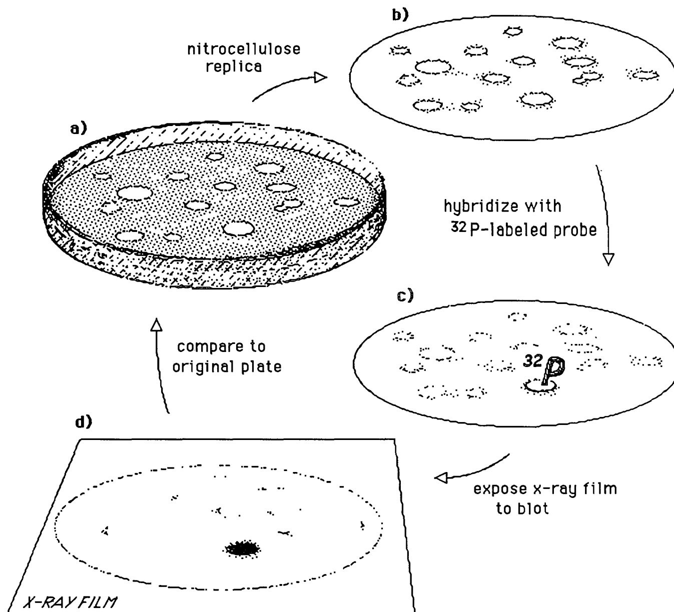

Figure 1-5. Plaque hybridization. **a**) An agar plate is shown that contains a bacterial lawn on which bacteria infected with recombinant bacteriophage have been plated. Each of the initially infected bacteria gives rise to a clear area, or plaque, on the plate, which results from the outwardly spreading infection and subsequent lysis of bacterial cells. Each of the plaques contains a multitude of identical phage. **b**) The phage transferred to nitrocellulose, lysed in place, and the phage DNA is denatured. **c**) The DNA replica is hybridized with a ^{32}P-labeled DNA probe, washed to remove unhybridized probe, and **d**) exposed to x-ray film to reveal plaques containing the clone of interest. The film is then compared to the original plate so that phage can be isolated and grown up for further study. Colony hybridization is carried out in essentially the same way.

hybridization, the nitrocellulose is removed and washed in buffers of increasing stringency to remove non-specifically bound probe from noncomplementary DNA sites and from the filter itself. The nitrocellulose is then covered with a sheet of x-ray film to reveal the plaques containing complementary DNA sequences as spots on the x-ray film. To recover phage clones identified by the hybridization procedure, the x-ray film is aligned with the original phage plate. Plaques lying under the autoradiographic spots are removed, and the phage are amplified on plates or in suspension culture. Usually, it is a good idea to rescreen the phage in these plaques, through several rounds of plaque hybridization, in order to isolate pure phage clones. Colony hybridization is similar to plaque hybridization; however, instead of probing for DNA sequences in plaques, the recombinant clones are grown up as isolated bacterial colonies on agar plates. These are then transferred to nitrocellulose, lysed in place, and hybridized in the same way that plaques are hybridized [71, 72].

Once a clone is isolated in pure form, it can be analyzed in a number of different ways, or can be used as a tool to isolate other clones. If one's interest is the gene itself, the next step may be to restriction map and sequence it. Genes will often extend beyond the borders of any single clone, and in these cases, it would be necessary to screen for additional clones that contain flanking DNA fragments. If one's interest is a genetic disease thought to be caused by an abnormality in a particular gene, clones representing both the normal and the mutant gene would have to be isolated. Since many inherited diseases are heterogeneous, in part because of different kinds of mutations in the same gene, it may be necessary to isolate clones from a number of different individuals (each requiring the construction of a separate genomic library).

GENOME ANALYSIS

Genetic diseases can result from single base changes (point mutations), from deletions or insertions of genetic material, or from chromosomal translocations [5]. One approach that can be used to identify the molecular defect underlying a genetic disease is to isolate the mutant protein (identified, for example, as an abnormal electrophoretic variant) and to characterize it by amino acid sequencing. Using this knowledge, the mutant gene can then be cloned and sequenced to define the genetic defect. This approach, however, is possible only for proteins that are expressed at levels that will allow their purification. It is also restricted to cases (usually point mutations) in which the defects are structurally minor (although they may be functionally profound). Mutations that prevent transcription or translation, for example, could not be analyzed by techniques that rely on the isolation of protein end-products. Furthermore, for most genetic diseases there is still no clue as to the proteins that may be affected.

The techniques of molecular genetics offer an alternative approach to identify mutant genes [6]. This involves the localization of the mutant gene to a particular chromosome by genetic analysis, and then to a region of that chromosome; this leads eventually to the isolation of the gene itself [73]. While this process may take years, it does provide a rational approach to what would otherwise be an impossible problem. Once the gene is sequenced, it can be "translated" on paper to determine the amino acid sequence of the encoded protein. This sequence itself may lend a clue as to the protein's function, and at the very least should provide an opportunity to find amino acid regions that have the potential to be antigenic. These peptides can be chemically synthesized [74] in an automated peptide synthesizer and used to raise antibodies that can be applied to the isolation and analysis of the normal (or mutant) protein.

RFLP linkage analysis

One of the first steps in isolating a gene is to localize the chromosome on which it lies. This can often be done by finding a genetic marker linked to the mutant gene, whose chromosome assignment is already known. A powerful

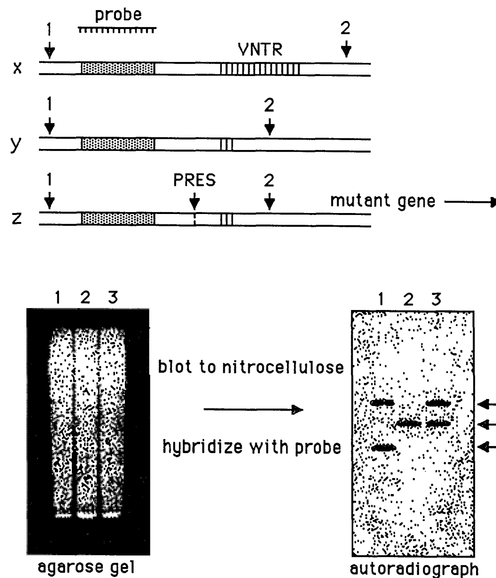

Figure 1-6. RFLP linkage analysis and Southern hybridization. Three polymorphic alleles are detected as RFLPs by hybridization to a probe (which could be any DNA sequence capable of revealing the polymorphism). Restriction enzyme digestion (arrows 1 and 2) gives rise to DNA fragments that differ in length in this region of the chromosome. The spacing between sites 1 and 2 is different in alleles x and y because of the number of copies of a repetitive sequence in a region containing a VNTR (variable number of tandem repeats). The spacing between restriction sites is different in alleles y and z because of the presence or absence of a single base change that creates or eliminates a PRES (polymorphic restriction endonuclease site). RFLP linkage anlysis has established that the mutant gene is co-inherited with the presence of the PRES (allele z). The genotype of individuals is determined by restriction enzyme digestion of their genomic DNA followed by Southern hybridization. Restriction-cut DNA appears as a smear after agarose gel electrophoresis and staining with the fluorescent dye ethidium bromide. Transfer of the DNA to nitrocellulose and hybridization with an RFLP probe reveals the sizes of the restriction fragments on each of the chromosomes. The autoradiograph shows that individual 1 is heterozygous, having alleles x and z; individual 2 is homozygous for allele y; and individual 3 is heterozygous, having alleles x and y. Individual 1 would be at risk for the inherited disease (or would be affected) if it were a dominantly acting mutation, and would be a potential carrier if it were a recessively acting mutation.

approach to this problem is to use restriction fragment length polymorphisms (RFLPs), first to localize the chromosome and then to narrow down the site [75, 76].

Polymorphisms are frequent variations in DNA sequence that are found between individuals or between homologous chromosomes within an individual. Polymorphisms may or may not affect the phenotype, but if they influence the pattern of restriction fragments in a detectable way, by changing the lengths of certain fragments, they can be used to map genes [77, 78]. There are two main types of RFLPs (figure 1-6). One is associated with single base changes that either create or eliminate restriction sites, yielding different-sized

DNA fragments. The other is associated with insertions or deletions between restriction sites, also yielding different-sized fragments. Although these types of variations generally occur in regions flanking (between) genes, they can be closely linked to mutated genes through families or within a particular population. Thus, RFLPs can be used to analyze the inheritance of a genetic disorder within a family when a probe for this region of DNA is available [10].

RFLPs are detected by Southern hybridization using probes (usually isolated by chance) that reveal the polymorphic restriction fragments (figure 1-6). The probes themselves are not usually related to the linked gene. The genomic Southern (named for E. Southern, the originator of the technique [79]) is carried out by digesting genomic DNA to completion with a restriction enzyme. This can generate on the order of a million different-sized fragments, which are then separated by gel electrophoresis. Because of the extremely large number of DNA fragments, the digest appears as a smear of DNA on the stained gel. Despite this, however, each DNA restriction fragment migrates according to its characteristic size; relatively small differences in the sizes of the polymorphic fragments detected by a probe can be resolved by hybridization. After electrophoresis, the DNA is transferred by blotting to nitrocellulose. The DNA is denatured during this process and binds to this solid support, giving rise to a replica of the separated fragments. The blot is then hybridized with a radioactive probe (some procedures make use of a nonradioactive detection system) and the hybridizing fragments are revealed by x-ray autoradiography [80].

RFLP linkage analysis might start by making use of RFLP-specific DNA probes for all of the chromosomes. The initial step would be to establish linkage (or co-inheritance) of an RFLP with the expression of the genetic disease. Linkage can be considered established by genetic analysis when, in a collection of families, certain statistical criteria are met. To date, at least 50 genetic diseases have been linked to chromosome-specific RFLPs [81]. After the chromosomal assignment has been made, other RFLP probes specific to the same chromosome are used to find a probe with a statistically more reliable linkage, in order to narrow down the distance between the probe sequence and the mutant gene itself. A probe that shows close linkage can then be used to screen a genomic library.

The clones that are isolated in these cases may not contain the disease-causing gene, but rather a segment of DNA that lies (perhaps still at a considerable distance) upstream or downstream from the gene of interest. These clones can be used, nevertheless, as a starting point on the chromosome to begin a systematic, step-by-step process to isolate successive, adjacent (but overlapping) clones until the gene of interest has been reached. This is carried out by a process known as chromosome walking [82, 83], in which overlapping clones are isolated by sequentially screening the genomic library with probes containing DNA sequences at the distal ends of each previous clone. It is also possible to jump or hop along the chromosome to make faster progress [84].

Identification of the gene itself—knowing when you have arrived—requires additional information about the expression of tissue-specific mRNAs, which can often be obtained by cDNA cloning.

Diagnostic approaches

RFLP linkage analysis can be used to predict the presence (presymptomatically) of an inherited disease [11, 85], as long as there are sufficient number of family members whose DNA can also be analyzed to establish linkage. This can be accomplished even when nothing is known about the biochemical basis of the disease, and before the gene is actually isolated. The disadvantage of this approach is that there is a certain chance of error, since linkage to, rather than identification of, the disease gene is being carried out, and there is always the finite possibility that the gene and marker may become unlinked by genetic recombination. It is also necessary, and not always possible, that the appropriate family members be available.

Isolation of a disease gene allows it to be analyzed at the sequence level to determine the nature of the molecular defect. This knowledge makes it possible to design diagnostic approaches to identify with certainty the gene in individuals carrying the mutation. It may be possible in some cases, for example, to identify a mutant gene by whether or not it can be cut by a certain restriction enzyme. While the likelihood is small that a mutation may be located in a convenient restriction site, there are several known cases (e.g., the insulin and β-globin genes) where point mutations responsible for abnormal gene function have occurred within restriction sites, thus eliminating them [86–88]. These can be detected by Southern hybridization using a probe for the affected restriction fragment; mutation of the restriction site would give rise to a longer fragment representing DNA sequences including adjoining restriction fragments.

Knowledge of the mutant sequence also provides an opportunity to synthesize a diagnostic oligonucleotide probe that can specifically distinguish between the normal and mutant alleles by Southern hybridization [89]. The probe, representing the normal DNA sequence, would span the region of the mutation. Hybridization of the probe to the blotted genomic fragments would allow detection of the normal but not the mutant DNA sequence, if hybridization conditions were used that only permitted stability of perfectly matched duplexes [90]. It is also possible to hybridize a labeled single-stranded oligonucleotide probe to denatured uncut DNA, followed by restriction enzyme digestion of the oligonucleotide-genomic DNA duplex. Cleavage of the labeled probe would take place if the duplex were perfectly matched, but would not occur if there was a base-pair mismatch caused by a point mutation. The cleaved oligonucleotide would be detected by gel electrophoresis [91].

The sensitivity of the allele-specific oligonucleotide/restriction-enzyme cleavage method can be increased significantly by the selective amplification of the desired gene sequence using an in vitro polymerase chain reaction (PCR)

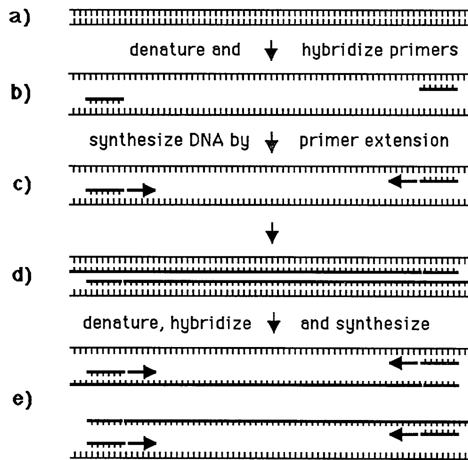

Figure 1-7. Polymerase chain reaction. Double-stranded DNA (**a**) in a region of a chromosome being tested is denatured into single strands (**b**) to which primer oligonucleotides are hybridized. DNA is then synthesized (**c**) in both directions by primer extension (arrows) giving rise to double-stranded DNA (**d**) containing one original strand and one newly replicated strand (heavy line). The cycle is then repeated by denaturation, primer hybridization, synthesis, etc., and results in up to a 200,000-fold (or greater) amplification (**e**) of the chromosomal region.

[92, 93]. The PCR amplification technique involves two priming oligonucleotides that flank the region of the mutation and are complementary to the opposite DNA strands (figure 1-7). The primers are annealed to the denatured DNA strands and extended by DNA synthesis. Repeated cycles of denaturation, annealing, and synthesis are carried out in a chain reaction, such that the newly synthesized strands are used as templates for further DNA synthesis, exponentially increasing the number of DNA copies of the target region. The process is facilitated by using a heat-stable DNA polymerase from *Thermus aquaticus* (Taq polymerase), because new enzyme does not have to be added after each round of denaturation [94–96]. This method permits a 200,000-fold or greater amplification of very small amounts of genomic DNA that can be isolated from as few as 100 (or fewer) cells, and can even go so far as to allow the analysis of a single gene in a single human sperm [97]. PCR has been used for prenatal diagnosis and carrier testing, and for the detection of somatic mutations thought to be responsible for the activation of proto-oncogenes and for the generation of chronic myeloid leukemia and acute lymphocytic leukemia [91, 94, 98–100].

GENE STRUCTURE AND EXPRESSION

Levels of regulation

Organisms have evolved an amazingly intricate and complex system of regulation to control and coordinate all of their functions, including embryonic

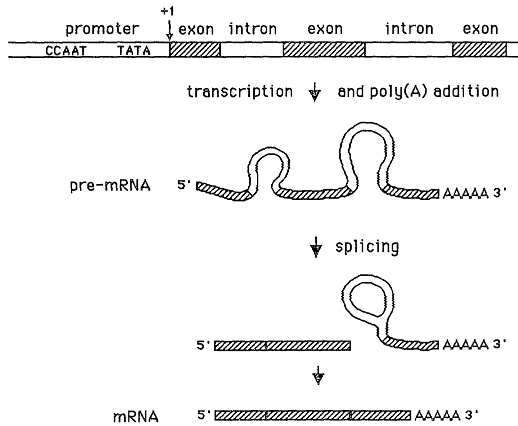

Figure 1-8. Gene structure and expression. The alternating exon–intron–exon structure of a eukaryotic gene is shown on the top line. The promoter region containing the CCAAT and TATA sequence elements is upstream of the transcribed region of the gene, which begins at the +1 nucleotide. The primary RNA transcript contains information encoded by exons (cross-hatched) and introns (open). After capping at the 5′ end and polyadenylation at the 3′ end, the intron sequences are removed from the mRNA precursor. The first splicing step is cleavage at the 5′ end of the intron and formation of a covalent bond between the intron 5′ end and a nucleotide within the intron, giving rise to a branched structure, or lariat intermediate. The second splicing step is ligation of the exons and removal of the intron.

and postembryonic development, metabolic homeostasis, immune surveillance, locomotion, and sensory and higher-order mental processes. Molecular biology is just beginning to enter these fields and, while still only in its infancy, is starting to reveal some of the genetic controls that orchestrate these fundamental life processes. Gene structure itself has proven to be far more complex than was imaginable only ten or so years ago. At one time it was believed, quite logically (and this was based on sound experimental evidence), that each gene is a relatively simple unit of information that encodes a unique protein. It is now quite clear that genes can be as simple as that, but that many (if not most) eukaryotic genes are mosaics of information that can give rise to several (or many) different species of similar but related proteins. This is made possible by the exon–[intron–exon]$_n$ structure of genes [10] and the alternative RNA processing mechanisms that follow their transcription.

A gene is delineated by the points at which transcription is initiated and terminated (figure 1-8). This defines the transcription unit itself. In addition, there are regulatory regions that flank the gene on its 5′ or upstream side (and sometimes on its 3′ or downstream side). The regulatory elements function to allow RNA polymerase to recognize the gene and to control where and when to initiate transcription [18]. All genes have at minimum a set of regulatory sequences that permits a basal level of RNA synthesis. In addition, there can be other regulatory elements that allow genes to be up-regulated or down-

regulated, or to be controlled in a developmental or tissue-specific manner [102]. Regulation does not end there, however.

After the primary RNA transcript is synthesized, it can then be processed into one of several alternative mRNA products, first by establishing where the 3′ end of the message will be, and then by choosing one of the several splicing pathways available to it [103]. Finally, the mature mRNA is transported to the cytoplasm, where it functions in protein synthesis. Again, regulatory mechanisms can come into play that will determine mRNA half-life and the rate of protein synthesis, to govern the levels of particular mRNAs and the levels of their translation products to fit the needs of the cell [104].

Intron–exon structure

Exons are gene regions encoding RNA sequence information that ends up in mature mRNA products. Introns are stretches of DNA within genes lying between exons. Introns are transcribed along with exons as segments of the primary transcript. They are removed during RNA processing and as a result are not found in the final mRNA product. Genes seem to fall into three categories: those without introns, those that have introns for no clear reason, and those that have introns for some obvious purpose.

There appears to be little or no size constraint on introns and exons (and on the genes themselves). Exons are often quite small, relative to introns. Thus, a gene with a fairly small protein-coding region and with only one (or no) intron can be very compact in size (on the order of hundreds of base-pairs in length), whereas a gene with a large number of relatively long introns can be spread out over a considerable distance (tens of thousands of base-pairs). A case in point is the α2(type I)collagen gene, which has been shown to contain over 50 introns [105]. Even more remarkable is the Duchenne muscular dystrophy gene, which has not yet been completely analyzed, but is thought to be on the order of 2000 Kb (2,000,000 base-pairs) in length [106].

Most introns lie within protein-coding regions of genes, and because of this, their transcripts have to be removed to generate functional, translatable mRNAs. The process by which introns are removed is called splicing [107]. This is actually a two-step reaction: first, the 5′ end of the intron is cleaved and simultaneously ligated to the branch-site nucleotide within the intron (giving rise to a lariat structure as an intermediate in the reaction), and second, the 3′ end of the intron is cleaved and the exon fragments are ligated together (figure 1-8). Splicing signals are present at every exon–intron (5′) and intron–exon (3′) border. These are relatively short sequences that designate the 5′ and 3′ ends of introns, and precisely specify the sites for this two-step cleavage and ligation reaction.

Each intron has to be removed with absolute fidelity. If even a single nucleotide were inappropriately added or deleted during the splicing of any of the introns within the coding region of a precursor, the translational reading frame of the resulting mRNA would be altered and the mRNA would be

incapable of producing a proper protein. (Either a termination codon would be encountered by the translational apparatus, causing premature termination, or a completely abnormal amino acid sequence would be generated, giving rise to a totally unrelated polypeptide. In fact, both of these would probably happen.) The thalassemias have been particularly instructive in this regard [108]. These are caused by mutations in the α- or β-globin genes that affect the levels (rather than the functions) of the globin chains. Just about every conceivable kind of genetic defect has been discovered in thalassemia patients, including point mutations in the critical splice-site sequences that block normal splicing, and point mutations in intron or exon sequences that actually create new splice sites from sequences that resemble but do not normally function in splicing. With this latter class of mutations, a choice of two alternative splices sites (one normal and one abnormal) is presented to the cell. Some but not all of the gene transcripts are spliced correctly, resulting in decreased levels (rather than a complete absence) of the globin chain.

Tissue- and cell-specific gene expression

Transcriptional controls

Prokaryotic (bacterial and bacteriophage) systems have been extremely valuable in elucidating some of the basic elements of genetic control. The important lesson that has been learned is that transcriptional regulation operates through DNA–protein interactions [109–111]. A paradigm for this is the lactose (*lac*) operon of *E. coli* [112], which is under the control of two oppositely acting regulatory proteins, the *lac* repressor and the catabolite gene activator protein (CAP) (also called the cyclic AMP receptor protein, or CRP).

When the enzymes encoded by the lactose operon are not needed, transcription is blocked by the repressor, which recognizes and binds to a DNA sequence (operator) at the 5′ end of the transcribed region. This prevents RNA polymerase from binding to the promoter and initiating transcription. When conditions change and the enzymes of the *lac* operon are needed (lactose is present and required by the cell), the repressor dissociates from the operator, giving RNA polymerase access to the promoter. At the same time, CAP (together with cAMP) associates with another DNA sequence (adjacent to the RNA polymerase binding site) to facilitate the binding of RNA polymerase and the initiation of transcription.

The important features of this dual control system are the presence of the positively and negatively acting regulatory proteins in the cell and their ability to sense and respond to the relevant signaling molecules. The repressor responds to increasing levels of lactose, and CAP responds to increasing levels of cAMP (brought about by decreasing glucose levels). These effector molecules interact allosterically with their respective regulatory proteins to increase (CAP–cAMP) or decrease (repressor–lactose) the affinity of these proteins for their corresponding DNA elements. The regulatory proteins are *trans*-acting

factors, so named because they move on and off the DNA elements they recognize. (CAP actually coordinates the regulation of a number of catabolite–gene operons [113], whereas the repressor is specific only to the *lac* operon.) In contrast, the DNA sequences in the promoter/operator region are called *cis*-acting elements because they are physically connected and relatively close to the genes they control. Bacterial cells are able to specifically and coordinately regulate the levels of a number of key metabolic enzymes at the level of transcription, by making use of *trans*-acting factors and *cis*-acting elements.

Eukaryotes also employ *trans*-acting factors and *cis*-acting elements for the regulation of gene expression, and while they utilize somewhat different strategies of genetic control, these regulatory systems may in some ways be considered variations on the same theme [114]. There are three classes of *cis*-acting regulatory elements characteristic of eukaryotic genes. Two of these lie upstream of the point at which transcription is initiated (in what might be called the promoter region). The third type, the so-called enhancer sequences, can lie anywhere in the vicinity of the gene.

Cis-acting elements adjacent to eukaryotic genes are now known to regulate transcriptional rates (both up and down) when associated with their corresponding *trans*-acting factors. Most protein-coding genes appear to have some combination of one, two, or all three types of *cis*-acting elements [115]. Thus, transcriptional regulation in eukaryotes (in a manner that is analogous to transcriptional regulation in prokaryotes) appears to be mediated by control regions that are composed of different combinations of promoter and enhancer elements, usually arranged in tandem, that seem to allow different regulatory factors to function coordinately [102]. An initiation complex is thought to form by the interactions of *trans*-acting factors with their DNA elements, and with each other via protein–protein interactions, to facilitate the binding of RNA polymerase to the appropriate sequence region to initiate transcription [114].

The three classes of *cis*-acting elements appear to have somewhat different functions. Just upstream of the point at which initiation takes place (designated the +1 site or cap site, so called because it codes for the first nucleotide of the transcript and is modified posttranscriptionally by the addition of the cap), there is often a sequence between approximately −25 and −30 (nucleotides upstream of the +1 site are given negative numbers) that helps to determine the exact start site for transcription. This element usually conforms to the sequence TATAAA, or something very close it, and as a result is named the TATA box [116].

Further upstream, usually around −50 to −150, is a region that can contain one of several sequence elements that are required for maximal expression. There can be a variety of elements in that region that are more or less specific to certain classes of genes. One of these elements, which has the consensus sequence CCAAT, is shared by a large number of genes. It is known to be the binding site for a *trans*-acting factor called either CTF (for CCAAT transcrip-

tion factor) or NF-1 (for nuclear factor 1) [117] that can stimulate transcription approximately 10–25 fold. Another element found in this region, which has the consensus sequence GGGCGG (called the GC box), is known to be the binding site for the promoter-specific transcription factor, Sp1 [118].

Enhancers are *cis*-acting elements that can be responsible for basal-level gene expression and for tissue-specific gene expression [119, 120]. They differ from promoter elements by being able to function in a characteristic position-independent fashion, and by being located upstream, downstream, or even within a gene (in one of the introns). Enhancers can also function at considerable distances from genes (up to several kilobases). The *trans*-acting factor AP-1 (for activator protein 1) is an enhancer-binding protein that regulates a number of genes, and is responsible for either basal level expression or induced expression, depending on the gene [121, 122].

The glucocorticoid receptor is an enhancer-binding protein that interacts with the glucocorticoid receptor element (GRE) located adjacent to hormone-responsive genes. Target cells, defined as such because they contain glucocorticoid receptor, are triggered by the binding of hormone and receptor in the cytoplasm. This interaction activates the receptor molecules, permitting them to bind to the GREs in the nucleus and to stimulate gene transcription [123].

Tissue-specific expression of the immunoglobulin genes is mediated by an enhancer [124–127]. Somatic rearrangement is required for the expression of the immunoglobulin genes [128]. This is an event that brings a variable-region gene adjacent to a constant-region gene to form a complete, transcriptionally active antibody-producing immunoglobulin gene. Only somatically rearranged immunoglobulin genes are transcriptionally active, even though each of the numerous variable-region genes (unrearranged and rearranged) has its own promoter. The unrearranged variable-region promoters are, by themselves, transcriptionally inactive. Rearrangement brings a single variable-region gene (together with its promoter) into juxtaposition with a constant-region gene and, as a result, into the proximity of an enhancer, located in one of the constant-region introns. This activates the promoter of the rearranged variable gene. In contrast, the unrearranged variable-gene promoters remain inactive because of the enormous distance along the chromosome, which places them too far away from the constant-region enhancer to feel its effects.

Some kinds of somatic mutations can activate genes inappropriately by bringing them under the control of other, more active loci. An example of this is the chromosome 8: chromosome 14 translocation associated with Burkitt's lymphoma. In this case the normally inactive c-*myc* proto-oncogene on chromosome 8 is translocated (reciprocally) to the active immunoglobulin locus on chromosome 14 [129]. The proximity of the immunoglobulin gene is believed to activate the c-*myc* gene, causing it to overproduce its otherwise normal product, and in doing so to drive the cell toward malignant transformation. Although the mechanisms involved in the activation of the c-*myc* gene have not yet been completely worked out, it is possible that the promoter has lost

a negative regulatory element or, alternatively, is responding to the nearby immunoglobulin enhancer [130].

Transcription factors are thought to regulate cellular events by being activated by signaling mechanisms. Target cells are able to respond to particular signals by virtue of having the necessary *trans*-acting factors (as directed, presumably, by prior events that instructed the cell to synthesize them). Although there is no direct evidence for this yet, it is currently thought that, in some cases, inducers of gene transcription may exert their effects by posttranslational modification (e.g., phosphorylation) of the relevant *trans*-acting factors, to affect their transcription-inducing or their DNA-binding activities [102].

Posttranscriptional controls

ALTERNATIVE CLEAVAGE AND SPLICING. The production of translatable messenger RNAs from eukaryotic genes requires a number of posttranscriptional processing steps, including the removal of intron sequences from the primary gene transcripts [18, 107]. For most genes there is one, and only one, pathway of messenger RNA production. This constitutive (or required) pathway generates a single species of mRNA from a single gene. Other genes (over 50 of this type are now known) follow a more complex pathway of messenger RNA production [103, 131]. In these cases, a single gene is capable of producing alternative primary transcripts, or alternative mRNA species from a single primary transcript. These mRNA species usually have some sequence information in common, and as a result produce different but related protein isoforms.

Sequence differences can be generated at either end of, or within, an mRNA (figure 1-9). A gene, for example, can have two promoters, and as a result can produce two different 5′ exons. The mouse α-amylase gene is an example. In this case, the two promoters are regulated in a tissue-specific fashion, one utilized in the salivary gland and the other in the liver [132]. Cleavage near the 3′ end of a primary transcript gives rise to the site at which the poly(A) tail is added to form the mature 3′ end of the mRNA. Pre-mRNAs can have alternative cleavage (and polyadenylation) sites that will result in the inclusion or exclusion of sequence information at the 3′ end. An example is the immunoglobulin μ heavy chain (IgM) gene, where alternative cleavage sites are chosen depending on the state of maturation of the immunocyte [133–135]. Cleavage at the downstream site in the resting lymphocyte adds an exon that encodes a sequence of hydrophobic amino acids. This sequence serves as a membrane-spanning domain that anchors the protein on the cell surface, where it acts as a receptor. Later in the development of the lymphocyte, cleavage of the mRNA precursor (produced from the same gene) occurs at the upstream site. This removes the portion of the mRNA that encodes the hydrophobic sequence, and results in an immunoglobulin that can be secreted from the antibody-producing plasma cell.

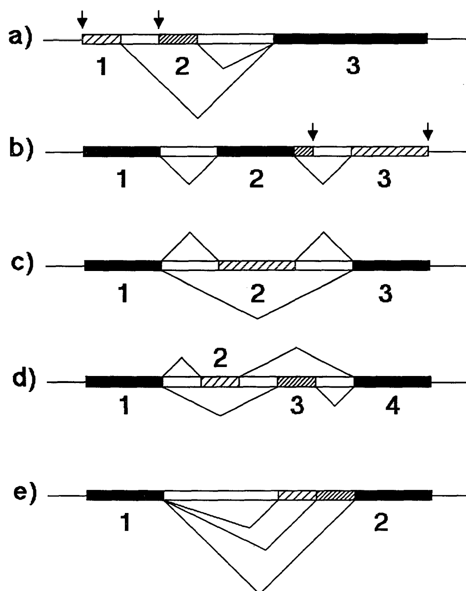

Figure 1-9. Alternative RNA processing. Five motifs are shown for generating different but related products from a single gene. Constitutively expressed exons are black, introns are open, and alternatively expressed sequences are hatched. Spliced ends are connected by brackets. **a**) Transcription can start at either of two points (arrows). Initiation at the leftmost arrow would result in exon 1 being spliced to exon 3, whereas initiation at the rightmost arrow would result in exon 2 being spliced to exon 3. An example of this is α-amylase. **b**) Cleavage and poly (A) addition can occur at either of two points (arrows). Cleavage at the leftmost arrow would result in an mRNA consisting of exons 1 and 2, where exon 2 is elongated by additional sequences (hatched); cleavage at the rightmost arrow would result in an mRNA consisting of exons 1, 2, and 3, where exon 2 is truncated. An example of this is IgM. **c**) An exon may or may not be spliced into the mRNA product. An example is αA-crystallin. **d**) Exon selection can be mutually exclusive. When exon 2 is spliced to exons 1 and 4, exon 3, is excluded; when exon 3 is spliced to exons 1 and 4, exon 2 is excluded. An example is troponin T. **e**) An exon may have several ends. An example is fibronectin.

The internal exons of primary transcripts can also be alternatively spliced. There are cases where a sequence that functions as an exon in one pre-mRNA molecule is removed in another (e.g., αA-crystallin [136]). In other situations, adjacent exons are alternatively spliced in a mutually exclusive manner (e.g., troponin T [137]). In both of these examples, one RNA's exon can be another's intron, and thus a clear-cut distinction between exons and introns can be somewhat difficult to make. More complex cases also exist, in which exons can be spliced together utilizing several different splice sites at one end or the other of introns (e.g., fibronectin [138]). This suggests that, rather than being considered absolute entities, exons and introns need to be defined operationally.

It should be emphasized that in all of these complex pathways, splicing

brings mRNA sequences together that are then translated into protein. Therefore, they have to be spliced together in such a way as to maintain their translational reading frames. Depending on the case, the proteins resulting from alternatively spliced mRNA precursors can have different amino-terminal, carboxy-terminal, or even internal amino acid sequences. As the degree of complexity increases, the number of different, alternative mRNAs (and potential protein isoforms) increases exponentially as a function of the number of exons that participate in the splicing reactions. The gene for the muscle protein, troponin T, has 18 exons. Eleven of the exons are constitutively spliced, five are alternatively spliced (in every possible combination), and two are spliced in a mutually exclusive fashion. The extraordinary result is that 64 different developmentally regulated protein isoforms are produced from a single troponin T gene [139].

Although the biochemistry of splicing has not yet been worked out in complete detail, it is becoming evident that the reactions involve a large number of RNA and protein cofactors [140, 141]. The tremendous burden on the cell to maintain introns within genes and to elaborate the splicing apparatus, and the enormous amount of energy that is expended to transcribe long stretches of RNA that are subsequently wasted, would suggest that splicing has some very fundamental role in cell function. Its contribution to the generation of protein diversity is now well established. However, it is also tempting to speculate that splicing plays an important regulatory role in the production of all mRNAs derived from genes with introns. Where genes give rise to alternative protein products, it is likely that the choices are not made at random, but are determined by the needs of the cell. Even in cases where only one protein is made from an intron-containing gene, it seems possible that the required splicing step may be a point at which a decision could be made as to whether processing will continue and ultimately give rise to a functional mRNA, or will not continue further.

MRNA STABILITY. The steady-state levels of mRNAs depend not only on their rates of synthesis (and processing), but also on their rates of decay. Individual mRNAs are known to have different cytoplasmic half-lives that range from only minutes to as long as days or even weeks [17]. Short half-lives allow mRNAs to be expressed transiently and to respond quickly to environmental signals. Long half-lives allow mRNAs to accumulate to high levels. Protein-synthetic capacity can be significantly amplified by stabilizing mRNAs, since mRNAs that exist for longer periods of time can produce more protein.

mRNA stability can be regulated to suit the requirements of the cell. Constitutively expressed mRNAs (that produce the so-called housekeeping proteins required of all cells) are now thought to be regulated primarily by mRNA stability [142]. Other mRNAs are known to be stabilized in response to hormones (e.g., vitellogenin mRNA [143]), cAMP (e.g., phosphoenolpyruvate carboxykinase mRNA [144]), and growth factors (e.g., type I procollagen mRNA [145], EGF receptor mRNA [146]). At least one mRNA (type I pro-

collagen mRNA) is known to be destabilized by hormone (dexamethasone) [147].

The biochemical mechanisms involved in regulating mRNA half-life are not well understood. The poly(A) tail (present on virtually all mRNAs) may contribute to mRNA stability, and may be involved in the process of regulation [148, 149]. Several mRNAs appear to be regulated by sequences in either the 5′ or 3′ untranslated region [142]. These regions operate as *cis*-acting elements, and function in response to *trans*-acting regulatory factors. One example is a class of transiently expressed mRNAs, including those for the c-*fos* and c-*myc* proto-oncogenes, interferon, and the lymphokine, GM-CSF [150, 151]. These mRNAs have short half-lives, and share an AU-rich sequence motif in their 3′ untranslated regions. The possibility that these mRNAs are under the control of a *trans*-acting factor is suggested by the observation that they are stabilized by the protein synthesis inhibitor, cycloheximide, which is thought to block the synthesis of a labile regulatory protein, thereby reducing its amount. Alternatively, inhibition of protein synthesis itself may more directly affect the stability of these mRNAs, which are thought to require active ribosome transit for normal turnover.

The stability of the mRNA for the transferrin receptor has been shown to be regulated by iron levels via a posttranscriptional mechanism [152]. Iron is thought to exert its effect through a *trans*-acting factor that recognizes a region of RNA secondary structure in the 3′ untranslated region of the mRNA. Tubulin mRNA is regulated by a *trans*-acting factor that senses the need for the mRNA and controls its level accordingly. The protein end-product of this mRNA, tubulin, appears to have a direct feedback role in this process [153–155]. Evidence suggests that as the level of unpolymerized tubulin subunits increases, the mRNA becomes destabilized.

The existence of multiple controls that act in concert at the levels of transcription, RNA processing, transport, mRNA stability, and translation allows gene expression to be regulated in a way to ensure a rapid response to physiological signals, to fine-tune the levels of end-products, and to provide high levels of expression of certain proteins under appropriate circumstances.

TOOLS FOR STUDYING GENE EXPRESSION

Methods of analysis

Rates of RNA synthesis

The regulation of gene expression can be examined directly by measuring the rate of transcription itself. Gene regulation frequently involves changes in transcriptional activity, as directed by the various *cis*-acting elements and *trans*-acting factors. Increased rates of initiation result in increased numbers of actively synthesizing RNA polymerase molecules, and in the end, increased numbers of mRNAs.

The rate of RNA synthesis can be measured by the incorporation of labeled

precursors into RNA. Hormone-stimulated cells, for example, will usually respond with a generalized increase in transcriptional activity, and will incorporate label into a variety of different mRNAs, as well as ribosomal RNA and transfer RNA. To answer questions about individual mRNA species, however, one must employ techniques such as nuclear run-on transcription to specifically identify the mRNA precursor (or gene) of interest. Nuclear run-on transcription is a technique that involves the labeling of RNA transcripts to high specific activity in isolated nuclei, permitting the subsequent analysis of these labeled transcripts by nucleic acid hybridization with gene-specific probes [156–158].

Isolated nuclei are permeable to nucleoside triphosphates (NTPs) (the immediate precursors of RNA). Therefore, it is possible to get the incorporation of high-specific-activity, ^{32}P-labeled NTPs directly into newly synthesizing RNA transcripts, without encountering the problems of getting a biosynthetic precursor specific to RNA into intact cells. At the time of isolation, nuclei are engaged in transcription, and thus have a number of active RNA polymerase molecules associated with certain genes. After isolation, the nuclei are incubated under conditions that support transcription. The RNA polymerases that were involved in transcription prior to isolation resume transcription and continue it (run-on) until they reach the ends of their respective genes. Thus, the nascent RNA molecules that were already initiated will get radioactively labeled, and the amount of label in any one RNA species will reflect the number of RNA polymerase molecules associated with the gene (the transcriptional activity) at the time of nuclear isolation.

The transcriptional activities of the genes being studied by nuclear run-on transcription are analyzed by blot hybridization. Blots are made on which cloned DNAs (or their antisense RNA transcripts) are affixed. (The blots are usually made with a slot-forming manifold, and as a result are called slot blots.) These are hybridized with labeled RNA from the run-on transcription step, and the amount of specifically hybridizing, labeled RNA is determined by autoradiography. The hybridization reaction is carried out with an excess of nucleic acid (cloned DNA or antisense RNA) on the blot, so that hybridization of the labeled RNA goes to completion. As such, the intensity of the autoradiographic signal on each slot reflects the number of transcripts on each of the active genes, and thus their relative rates of transcription.

mRNA levels

Transcription is only the first step in the production of mRNA, since post-transcriptional processing and mRNA stability can also determine the levels of cytoplasmic mRNAs. Thus, to evaluate gene expression at the level of functional gene products, it is often necessary to measure the steady-state levels of specific mRNAs. In fact, this is often the starting point when analyzing gene expression. If a change in steady-state mRNA is observed in a certain developmental sequence or following the induction of gene expression

by a physiological stimulus, the rate of gene transcription can then be examined by nuclear run-on analysis to determine whether the change in the amount of mRNA is caused by transcriptional or posttranscriptional mechanisms.

The two most common techniques for measuring mRNA levels are dot blot hybridization and Northern blot hybridization [80, 159]. Both of these methods involve the isolation of unlabeled RNA from the cell or tissue of interest, followed by its immobilization on nitrocellulose and the subsequent hybridization of this RNA with a labeled probe. (This is in effect the opposite of the run-on transcription analysis described above in which total nuclear RNA is labeled and hybridized to an unlabeled probe sequence immobilized on nitrocellulose.) The only difference between a dot blot and a Northern blot is that RNA is applied to the nitrocellulose either as a small circle (usually with a dot-forming apparatus) or after gel electrophoresis to resolve mRNAs by molecular weight. (The term Northern blot is derived from its similarity to the Southern blot, and the concept to some that RNA is the opposite of DNA, thus requiring that it be given a name reflecting this diametric polarity. Whether Northern is capitalized or not is optional.)

The Northern blotting procedure starts with the electrophoresis of RNA on an agarose gel to resolve mRNAs by molecular weight (figure 1-10). The RNA is then transferred to nitrocellulose [160] by capillary action, and the blot is hybridized in a sealed plastic bag with a ^{32}P-labeled (nick-translated DNA or SP6-generated antisense RNA) probe. Many abundant and moderately abundant mRNAs can be detected (with high-specific-activity probes) in samples of total cellular RNA, while detection of the less-abundant mRNAs may require the purification of poly(A)$^+$ mRNA. Messenger RNA usually comprises less than 5% of the RNA of the cell. (The 18S and 28S ribosomal RNAs are by far the most prominent RNA species resolved on agarose gels, and usually the only discrete RNA species observed by staining these gels for RNA. In contrast, the mRNA population is very heterogeneous in size and would appear as a faint smear through the gel lane, if it were visible at all after staining.) One round of oligo(dT)-cellulose chromatography can enrich for poly(A)$^+$ sequences approximately ten-fold (two or three rounds can provide further enrichment), and thus can allow a corresponding increase in the amount of mRNA that can be loaded onto a gel lane. Although there is a practical limit to the amount of RNA that can be applied to these gels, which places a limit on the sensitivity of the technique, Northern blotting should be able to detect mRNAs that are in the range of only a few copies per cell.

Northern hybridization can be used as a semiquantitative approach for the comparison of one sample to another, if equal amounts of RNA are applied to each gel lane. Hybridization with the labeled probes is carried out in probe excess (with respect to the mRNA sequences being analyzed) in order to saturate the complementary RNA sites on the nitrocellulose. After autoradiography (figure 1-10), the hybridization signals can be quantitated by densitometry and compared to one another and to standards.

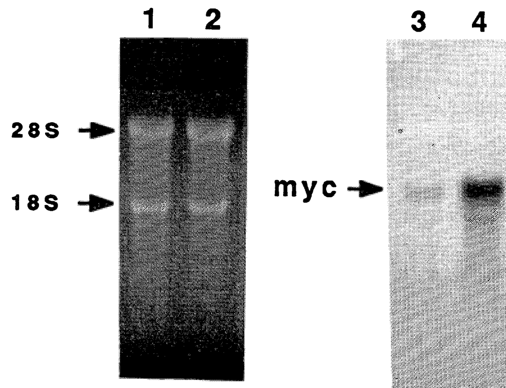

Figure 1-10. Northern hybridization. Two samples of total kidney RNA (lanes 1 and 2) were isolated and electrophoresed on a denaturing agarose gel, and the gel was stained with acridine orange. The 18S and 28S ribosomal RNAs are by far the most prominent bands, since 95% or more of the RNA in a cell is in these RNA species. (The appearance of these bands can serve as an indication of the integrity of the RNA preparation. There are equimolar amounts of the two RNA species, and since 28S rRNA is approximately twice the length of 18S rRNA and is also more susceptible to degradation, there should appear to be about twice the amount of 28S as 18S if the RNA preparation is intact. These two bands, in addition, can serve as convenient size markers). The gel was blotted to nitrocellulose and hybridized with a ^{32}P-labeled probe to determine the level of expression of the c-*myc* proto-oncogene in each of the two samples (lanes 3 and 4). The autoradiograph of the blot revealed that c-*myc* mRNA was induced to a much higher level in the sample on the right.

While it is oftentimes faster and more convenient to use the dot blot procedure, since more samples can be compared on the same blot, there is also the very real possibility that background or nonspecific hybridization will not be recognized. Therefore, it is advisable whenever dot blots are employed to first confirm the specificity of each probe by Northern analysis. Northern blots also have the advantage that mRNA size can be determined (or confirmed), and that tissue-specific size differences or multiple mRNA isoforms can be resolved from one another. Northern blots can also be stripped of hybridized probe and rehybridized with other probes that are also being investigated, or that may serve as internal controls.

cDNA +/− screening

Analysis of gene expression by transcriptional run-on or Northern blotting depends on having cloned DNA sequences to use as specific hybridization probes. To start out this way may require having some prior knowledge about what is expected to happen in the cell, a specific testable hypothesis, and the right clones.

If it is believed that differences in gene expression exist between different cell types or in the same cell under different conditions, but it is not known what

genes are differentially expressed, it should be possible to find these differences by cDNA +/− screening [161]. For example, if a particular signaling pathway is being investigated, one might be interested in knowing what genes are expressed in association with the responses that are elicited. Alternatively, if a developmental sequence is being investigated, or if normal and abnormal conditions are being compared, one might be interested in knowing what genes are responsible. As these may involve pathways (or conditions) about which little is known, or may involve tissue-specific or stage-specific gene expression that has not yet been characterized, it may be necessary to use methods that do not depend on having precloned sequences.

The principle behind the +/− cloning method is to compare mRNA populations (e.g., in cells that are induced and uninduced) by utilizing a cDNA library specific to one population that is then differentially screened with labeled cDNA probes representing the two mRNA populations. Most of the mRNAs in the two populations will be the same. The objective is to find the ones that are different. A cDNA library constructed with the mRNA from the induced cells, for example, will contain clones for the mRNAs that are differentially expressed upon induction, as well as a large number of clones that are not unique to induced cells. If this library is screened with a population of labeled cDNAs that is made to the same mRNA population used to make the library, every clone (theoretically) should hybridize. If, on the other hand, the library is screened with labeled cDNAs that are made to the mRNAs in the uninduced cells, some of the clones (representing induced mRNAs) will not hydridize. These are the clones being sought.

In practice, the cDNA library is plated out for screening, and duplicate nitrocellulose replicas of each plate are prepared for hybridization. The cDNA probes are made by synthesizing first-strand cDNA copies of the mRNAs in the two populations in the presence of ^{32}P deoxynucleoside triphosphates to label the cDNAs. Each of the duplicate nitrocellulose replicas is then hybridized with a different cDNA probe population, and the hybridization is analyzed by autoradiography. The autoradiographs should line up with each other and with the original plate from which the replicas were made. If a hybridization signal is found on one film (hybridized with the induced cell cDNA) but not the other, it would be a candidate for a cDNA clone representing an induced mRNA.

The relative number of cDNA clones in the library specific to a particular mRNA should correspond to the abundance of that mRNA. Therefore, the more abundant the induced mRNA, the more clones in the library, and the easier the mRNA should be to detect. cDNA +/− screening can also be sensitive to quantitative changes in mRNA levels, and not simply to their presence or absence. Once positive clones are identified, they can be isolated and analyzed in any number of ways to determine the proteins they represent and the roles these proteins have in cell function.

Localization

Virtually any cell, tissue, organ, or developmental stage is amenable (in theory, if not practice) to analysis by the techniques of molecular biology. This has been made possible in large part by having highly specific nucleic acid and antibody probes. The techniques for the isolation and purification of intact RNA are now fairly standard and can be applied to a variety of tissues. These RNA preparations can be used to construct cDNA libraries, which can be screened in a number of ways to isolate tissue-specific clones. Once the clones are isolated, sequence information can be obtained for the preparation of antibodies. The clones can also be used to investigate gene expression in the tissue of origin, and in other cell types, tissues, and organs.

If cDNA clones and antibodies are utilized in conjunction with histological approaches (in situ hybridization and immunohistochemical localization), almost any cell can be studied. In situ hybridization can be used (semiquantitatively) to assess steady-state mRNA levels in individual cells [162] or in tissue sections [163]. Although there are a variety of approaches, it is now standard to use antisense RNA probes. These are usually labeled with ^{3}H or ^{35}S NTPs, rather than ^{32}P NTPs, for greater microscopic resolution. Tissue sections are fixed and prepared for hybridization, incubated with the labeled probe, washed and treated with ribonuclease to remove unhybridized (but not hybridized) probe, and dipped in liquid autoradiographic emulsion. After exposure, the slides are developed, stained, and examined for silver grains over the tissue. The probe hybridizes directly to mRNA sequences, which can be present in individual cells at an abundance of only a few hundred copies up to thousands of copies (and higher) per cell.

Experimental systems for studying gene expression

Studies of gene expression in vivo in higher organisms have made use of a variety of elegant model systems, such as the sea urchin, Drosophila, amphibians, and the mouse, to identify genes that are active and expressed during embryogenesis, or in specialized cells in the adult [164]. Many of the questions being asked have to do with the role of the genome in controlling organismic development and cell function—the role of maternal RNA in early development, differential gene activity in the early embryo, and genetic control of pattern formation and morphogenesis. These types of studies require an understanding of gene function itself. While a great deal of our knowledge is based on biochemical approaches to characterize the systems, and on genetic approaches to decipher their functions, it has also been important to have in vivo model systems in which gene expression can be experimentally manipulated. Examples include hormone stimulated gene expression [165, 166], metal-induced [167] and heat-shock (or stress-) induced gene expression [168], and organ regeneration (liver and kidney) [169, 170].

The responses of cells to experimentation in vivo (induction or insult) take place in the arena of multilevel physiological signaling pathways and feedback

loops, and in the more complete context of cell–cell contact and complex tissue interactions within the intact (or semi-intact) organ. The liver that is experimentally induced to regenerate by partial hepatectomy, or the kidney that is induced to undergo compensatory hypertrophy by contralateral uninephrectomy, responds with increased cell growth. Of equal significance is the fact that in both cases the organs stop growing when they have reached some critical size or capacity. This is a phenomenon that will only occur in vivo. And at best we can only guess about the genetic events that may be responsible for these exquisite controls.

There are a large number of in vitro systems for the experimental analysis of gene function. All suffer the disadvantage that they are only close (and not identical) to the in vivo state. However, their convenience far outweighs this drawback. It is well recognized that cells take on new characteristics (almost immediately) upon being placed in culture [171], and that they can easily adapt to culture conditions to the extent, in some cases, that they hardly resemble their parent cell. Whether this is a manifestation of changes imposed on the cells by the culture conditions, or the selection of cells that become mutated and gain an advantage, is not clear. However, it is known that these changes in phenotype are a reflection of changes that occur in the expression of the genes. Despite this, cells in culture also retain many of their original characteristics and often express differentiated cell functions, such as regulated transport and hormone responsiveness [172]. Cells in culture utilize established biochemical pathways, and thus it is presumed that what is learned about molecular control mechanisms in vitro should be relevant (in some context) to mechanisms at work in vivo.

Cells in culture can be manipulated in any number of ways. They can be induced to grow or to differentiate [173]. In many of these cases, the response can be analyzed at the gene level by quantitating the amounts of particular (induction-specific) mRNAs by Northern blot hybridization, by carrying out transcription studies, or by identifying the induced genes by +/− cDNA cloning. Once genes have been identified by this type of analysis, their functions can be tested in vitro in the same cells, or in vivo by looking for their expression (and action) in the original cell type.

Gene function itself can be studied by the experimental manipulation of the gene of interest, followed by its (re)introduction into cells, where its expression can be monitored [36]. There are three basic objectives to these types of studies: first, to mutate putative control regions to test their functions; second, to mutate translated regions to analyze the functions of amino acid sequence domains; and third, to express (mutated or unmutated) genes to determine their roles in the context of other cell functions.

Genes can be introduced into cells by a variety of means. As discussed above, the simplest and generally most reliable techniques are calcium phosphate and DEAE-dextran-mediated transfection. DNA (to be expressed) is presented to cells in culture. These cells (transiently) express the genes that

were taken up, while a small fraction of cells will integrate the foreign DNA into their genomes and become transformed. Such stably transformed cells are useful for long-term studies of gene expression. DNA (also mRNA and protein) can be introduced into cells by microinjection. This is fairly simple with oocytes, because of their large size. Amphibian oocytes will support the functions of the foreign DNA by transcribing it, translating its mRNA, and processing the protein product [174]. Alternatively, mRNA can be injected directly into the egg cytoplasm, where it will be translated into a protein product that can be assayed immunologically or by monitoring its function [175].

Transgenic animals can be created by the introduction of DNA into mouse oocytes [176]. This DNA is incorporated into chromosomal regions at random, and is expressed in the developing embryo and at later stages in a variety of tissues and organs. A number of eukaryotic genes have been incorporated into transgenic animals, most notably the human growth hormone gene, whose expression was dramatically demonstrated by the larger size of the mice that received the gene [177]. Transgenic animals expressing the SV40 large T antigen gene (the transforming gene of the virus) have been shown to develop neoplasias in a number of different tissues and organs [178], including the formation of cysts in the kidney [179].

Tissue-specific expression of *trans*-genes requires that they be linked adjacent to (most, if not all) of the necessary *cis*-acting regulatory elements. In some cases, the objective may be to place a gene that is not normally expressed in a particular tissue under new control, so that the function of that gene can be studied in its new context. The c-*myc* oncogene, under the control of the mouse mammary tumor virus promoter (which itself is under hormonal control), has been shown to cause tumors when expressed in the mammary glands of lactating females [180]. The construction of genes that can be expressed in a tissue-specific fashion in transgenic animals will be an important step toward the development of in vivo animal models for human genetic diseases, and it appears that the molecular techniques are now at a stage where they can be realistically applied to this concept.

CONCLUSIONS

Our current understanding of the molecular basis of cell function would not have been realized without the development of recombinant DNA technology. The ability to clone and manipulate genes, to analyze these genes by DNA sequencing, and to study their structure and expression by nucleic acid hybridization has given rise to an explosion in our knowledge about the molecular control mechanisms involved in the replication of the genetic material, the biogenesis of messenger RNA, the synthesis of proteins, and potentially, any cell process that involves a DNA molecule, an RNA molecule, or a protein molecule. Every signaling mechanism, every cell–cell interaction, every metabolic event, and in fact every aspect of organismic biology is rooted in the

genetic material. And while a complete understanding of these intracellular and extracellular functions will require approaches that are outside of molecular biology, they will also depend, in part, on what the DNA itself can reveal.

Our current state of knowledge (and enough time) should permit us to isolate almost any gene to which a function or a phenotype can be attributed. Genes can be localized to specific chromosomal regions by mapping techniques, and when these techniques are combined with library construction and screening, genes can be physically isolated, subjected to a scrutinizing molecular analysis, and manipulated to understand their functions. We now know that genes are controlled by regulatory elements, that gene transcripts are processed in constitutive or alternative ways, that mRNAs are stabilized or destabilized, and that all of these processes (at all three levels) are under the control of *cis*-acting elements and *trans*-acting factors.

Gene expression can be investigated by hybridization, by protein analysis, and by functional studies that require the introduction of expression-competent genes into cells or animals. Gene activity itself can be examined by transcription in isolated nuclei, followed by hybridization of the labeled transcripts to cloned probes on nitrocellulose; levels of mRNAs can be examined by Northern blot hybridization where unlabeled cellular RNA is resolved by gel electrophoresis, transferred to nitrocellulose, and hybridized with labeled, gene-specific probes. The outcome of these approaches, when applied to a particular experimental system, is to learn more about what regulates the activity of genes and the levels of mRNAs, and how they respond to signals from within the cell, and from without.

One of the most pressing problems facing molecular biology today is to understand and to treat (or prevent) inherited disease. Our current knowledge indicates that genetic diseases can be very heterogeneous. They can affect virtually every step in the pathway from gene transcription to protein function. The thalassemia mutations, for example, which affect the α- or β-globin genes, can disrupt gene structure, promoter activity, RNA processing, mRNA stability, and translation. In fact, the thalassemias actually represent an assortment of mutations that are present in a large and varied group of individuals, who manifest the disease to different degrees with more or less severity. In contrast, sickle-cell anemia represents a unique mutation, caused by a single A-to-T transversion in the sixth codon of the β-globin gene (converting a glutamic acid to a valine in the β-globin polypeptide). Despite the fact that this is a discrete mutation, however, the prognosis for individuals with sickle-cell can vary considerably depending on their genetic background, as illustrated by the following cases.

Nearly one third of American blacks have α-thalassemia, a condition that usually arises from the deletion of one (or more) of the four α-globin genes. In its mildest and most common form, α-thalassemia is symptomless. In fact, α-thalassemia inherited concurrently with sickle-cell anemia can actually ame-

loriate the severity of sickle-cell disease, probably by lowering the intracellular hemoglobin S concentration and thus retarding the sickling process [181, 182]. In contrast, hemoglobin C results from a mutation of the same codon in the β-globin gene that causes sickle-cell anemia (converting the glutamic acid to a lysine rather than a valine). When inherited as a homozygous condition, hemoglobin C results in a relatively mild hemolytic anemia (in contrast to sickle-cell anemia). However, when this mutation is inherited together with sickle-cell trait, as a compound heterozygous state, it results in hemoglobin SC disease, which, while milder than sickle-cell anemia, is more severe than sickle-cell trait alone [5].

These cases show us that an inherited disease (such as thalassemia) can itself be heterogeneous (there are numerous ways to affect the levels of the globin chains). In addition, the severity of a disease can either be increased (hemoglobin SC) or decreased (sickle-cell and α-thalassemia) by the co-inheritance of two mutant alleles. It hardly needs to be said that an understanding of the causes of genetic diseases and the complex interactions of multiple alleles will require a molecular dissection of all of the genes involved, and that one of the foremost objectives of research in molecular biology should be to solve these problems.

While it is clear that the use of molecular approaches will accelerate our understanding of genetic disease, it should also be recognized that efforts to understand genetic disease should accelerate our understanding of basic cell function. The experimental geneticist creates mutations in the laboratory in order to pick apart and piece together cell structures, metabolic pathways, and developmental sequences. However, the natural mutations that are the antecedents of genetic disease can reveal a great deal about cell function that we would otherwise not understand [183]. The oncogenes, for example, and their normal counterparts, the proto-oncogenes, were discovered by investigating the tumors they caused. An elucidation of the role of these genes in cellular transformation has now provided a clearer picture of the biochemical basis of abnormal cell growth. Of equal significance is the fact that we also have a window on the signaling pathways that involve growth factors, their receptors, intracellular transduction systems, and the nuclear proteins that together function to regulate normal cell growth. If follows that, as other genetic diseases are investigated and solved, new windows will be opened to the cell.

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NOTES

[1] According to Webster's Dictionary, the term *molecular biology* was coined in 1938.

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2. APPROACHES TO THE DIAGNOSIS OF RENAL GENETIC DISORDERS USING DNA ANALYSIS

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Since the molecular structure of DNA was described by Watson and Crick in 1954, there have been remarkable advances in understanding the structure and function of eukaryotic genomes. Teachniques have been developed to manipulate genes, to reproduce (clone) them in large number, and to analyze them for alterations from the normal state, in individuals with genetic diseases. Assignment of normal and mutant genes to the human chromosomal map has enabled the diagnosis of genetic diseases, even in cases where the basic biochemical defect underlying the disease in question remains unknown.

These advances are usually applicable to the analysis of the single-gene disorders, i.e., those genetic conditions in which there is a Mendelian pattern of inheritance of the disease phenotype. For many of these autosomal dominant, autosomal recessive, or X-linked conditions, the gene responsible for the disease phenotype has been identified and cloned. In some cases, mutations causing disease have been identified at the DNA sequence level. The mutations may be caused by gross alterations in the DNA (such as deletions, insertions, or rearrangements), or by point mutations altering a single or a few nucleotides in the DNA sequence. For some Mendelian disorders, the disease gene of interest has been localized on the human gene map by means of genetic linkage studies with a gene or DNA segment whose chromosome location is known. The best example of a condition affecting the kidneys in which the map

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location is known, but the basic genetic defect is not, is the autosomal dominant adult form of polycystic kidney disease, linked to the alpha-globin locus and other DNA marker loci on human chromosome 16 [6].

Thus, for a disorder to be diagnosed by molecular genetic methods, one of two criteria must be met: 1) the gene causing the condition must be known and cloned, or 2) a closely linked gene or DNA segment must be identified. If at least one of these conditions is met, the diagnosis of a genetic disease by molecular techniques may be accomplished by either of two basic approaches: direct detection of the mutation, or indirect analysis of the region around the mutant locus by means of linked genetic markers.

The basic tools of the molecular diagnostician are the restriction endonuclease (or restriction enzyme) and gel electrophoresis. DNA isolated from individuals at risk for a specific genetic disorder is cut with restriction enzymes that cleave the DNA at specific *recognition sites* specified by a precise series of deoxyribonucleotides [7]. Gel electrophoresis is used to separate the DNA fragments by size; smaller fragments migrate further into the gel in a specified period of time. The fragments are then transferred from the agarose gel onto a solid support such as a nitrocellulose or nylon filter and exposed to a radioactively labeled DNA probe, which will hybridize to homologous fragments on the filter. The filter is placed on x-ray film, and fragments homologous to the probe appear on the film through the process of autoradiography; their position on the film reflects the distance they migrated into the agarose gel during electrophoresis, and hence reflects their size. The general term applied to this methodology is Southern hybridization, named not after a direction on the compass, but after Edward Southern, who first described the technique [8]. Alterations in the size of the restriction fragments revealed by hybridization to a specific gene probe may be a consequence of either common variation in the presence or absence of restriction recognition sites (polymorphism) or alteration of the DNA by a mutation that has functional implications for the gene product.

For direct analysis, the precise gene affected by the mutation must be known and cloned. The mutation may be detectable, either as a gross alteration of restriction fragments on Southern blot analysis, as would be the case in a deletion or large rearrangement, or by alteration of a restriction recognition site. Either of these processes changes the size of the DNA fragments generated by restriction endonuclease digestion of the gene in question. Alternatively, allele-specific oligonucleotide (ASO) probes, homologous to either the mutant or normal sequence, may be constructed. Under the appropriate conditions, these probes will hybridize exclusively to the allele to which they are homolgous.

For indirect analysis of the mutation by gene linkage methodology, polymorphic markers within or flanking the mutant gene may be used, or polymorphisms associated with a closely linked gene or DNA segment may be used to identify the mutant allele in a family [9, 10].

Other methods that are being used to detect mutations in the research setting, but are not yet in use for clinical diagnostic purposes, include chemical cleavage of heteroduplexes between mutant and normal DNA, RNAse cleavage of heteroduplexes between "normal" cDNA probes and mutant RNA [12], and denaturing gradient gel electrophoresis, which separates DNA fragments according to their base composition rather than their size [13].

DIRECT DETECTION OF MUTATIONS: DELETIONS, INSERTIONS, AND REARRANGEMENTS

Gross alterations of genes (those involving more than 100–200 base-pairs) are the easiest to detect by molecular diagnostic methods. DNA from the person at risk is digested with restriction endonucleases and subjected to Southern hybridization using a radioactively labeled probe from the gene or DNA segment of interest. Deletion of a portion of the gene, or insertion of a new fragment of DNA within the gene, or a rearrangement within the gene of interest will alter the size of the restriction fragments seen on Southern blots. The level of sensitivity for these alterations is between 100–200 base-pairs. Alterations affecting fewer base-pairs may not be detected by routine methods.

Tandemly duplicated genes such as the growth hormone and alpha-globin loci are at particular risk for gross rearrangement, which occurs as a result of faulty pairing and recombination resulting in unequal cross-over during meiosis. Genes not tandemly duplicated are occasionally mutated as a result of gross rearrangements, but (with few exceptions) this phenomenon is much less frequent, accounting for 1%–5% of mutations in these genes. For example, deletions or rearrangements in the beta-globin gene account for only about 1% of beta-thalassemia alleles [14], and fewer than 5% of patients with hemophilia A have gross rearrangements of the factor VII gene [15–18].

Example 1: The autosomal dominant condition in which Wilms' tumor (WT) presents with aniridia, gonadoblastoma, and mental retardation (WAGR) is associated with constitutive deletions of chromosome 11p13 [19] (figure 2-1). The catalase gene (*cat*) maps to 11p13 proximal to the WT locus; the gene encoding the beta subunit of follicle-stimulating hormone (FSHB) maps to the same region but distal to the WT locus [20]. Puissant et al. [21] described a patient in which the de novo chromosomal translocation 46,XY,-t(5,11)(q11;p13) was accompanied by a new mutation causing the WAGR complex. Southern blot analysis using probes for the *cat* and FSHB loci demonstrated a haploid dose of the *cat* gene in DNA from this patient, resulting from deletion of one allele. FSHB was present in diploid number. Thus, in this case, molecular methods were able to diagnose a microdeletion that was not cytogenetically visible.

Example 2: The fourth component of complement (C4) is encoded by duplicated genes, C4A and C4B, linked to the HLA complex on human chromosome 6 [22]. The absence of gene expression at these loci is called the C4 null phenotype, and is associated with a predisposition to systemic lupus erythe-

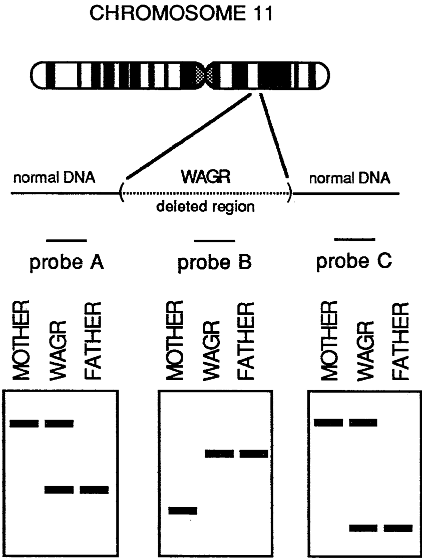

Figure 2-1. RFLP analysis of a proband with WAGR syndrome and his unaffected parents. Both parents are homozygous at loci within the proband's deleted region, and with probes proximal (A) and distal (C) to the region. The proband has inherited an allele from each parent for probes A and C, but only paternal allele for probe B. Thus, the father's B allele is deleted from the proband's DNA.

matosus (SLE) [23]. The C4A null phenotype is most often found with the HLA-B8, DR3 haplotype and results from deletion of the C4A locus [22], most likely as a consequence of unequal crossing over between the duplicated C4 loci.

DIRECT DETECTION OF MUTATIONS: POINT MUTATIONS

Mutations affecting a single or a few nucleotides are called point mutations and are a common cause of genetic disorders. Such mutations can be detected directly by two methods: restriction analysis or oligonucleotide probe hybridization.

Restriction endonuclease analysis is useful in the direct detection of point mutations only if the mutation alters a recognition site for a restriction enzyme. If a site is removed, or a new one created by the mutation, the restriction enzyme recognizes the sequence generated (figure 2-2A). Only about 5%–10% of mutations can be detected in this way [14].

Oligonucleotide probes provide an alternative method for the direct detection of point mutations (figure 2-2B). If the sequence of the mutation is known, a pair of oligonucleotide probes, precisely homologous to the normal and mutant sequences, may be synthesized. These probes are then hybridized

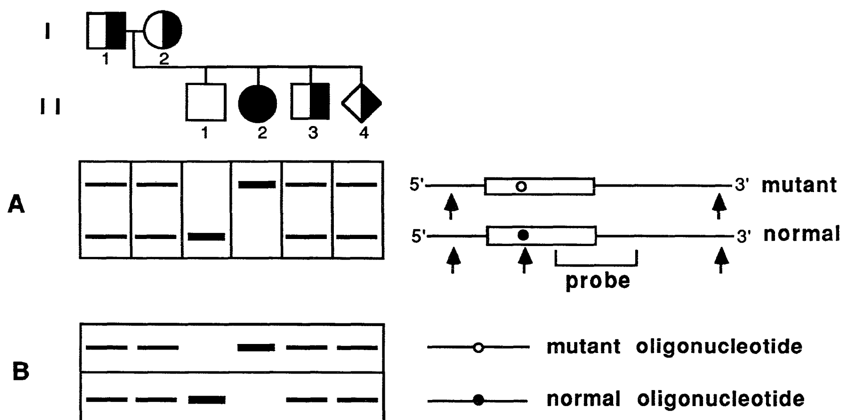

Figure 2-2. DNA diagnosis of an autosomal recessive mutation that disrupts a restriction endonuclease cleavage site. The parents (I) are both heterozygous for the mutation, and each has one allele with the normal site and one allele that lacks the normal site. Their affected daughter (II-2) inherited both mutant alleles. The fetus (II-4) is a heterozygote, and hence will be a carrier but not affected.
A. The white boxes represent the gene responsible for the recessive disorder in this pedigree. The black circle represents the normal nucleotide sequence and the mutation is indicated by the open circle. Arrowheads represent restriction endonuclease cleavage sites. Note that the mutant sequence eliminates a restriction endonuclease cleavage site, which is normally present.
B. DNA diagnosis of the same autosomal recessive disorder, the same family, using allele-specific oligonucleotides (ASO). The mutant allele, which is not cut by the restriction endonuclease (A), hybridizes to the "mutant" ASO, and the normal allele hybridizes to the "normal" ASO. DNA from heterozygotes hybridizes to both ASO probes.

to genomic DNA that has been digested by restriction enzmyes and subjected to electrophoresis and transferred to a nitrocellulose or nylon filter [24, 25]. Under optimal conditions, the "normal" oligonucleotide probe will hybridize only to DNA containing the normal sequence, and the "mutant" probe will hybridize only to DNA containing the mutant sequence. The major limitations of the oligonucleotide probe method are 1) the precise sequence of the mutation to be detected must be known—in many disorders, the mutation will be different from family to family; 2) the technique requires an experienced, highly specialized laboratory; and 3) in many cases, the mutant sequence will be different for the two alleles of a homozygote for a recessive disorder; these individuals are most often compound heterozygotes rather than true homozygotes for a single mutation. Nonetheless, oligonucleotide analysis is extremely useful for diagnosing those disorders in which one or very few sequence variations account for the majority of mutations.

A new technique with many applications to the detection of point mutations is the polymerase chain reaction, or PCR. Amplification of any DNA region of interest (provided sequences 5′ and 3′ to the region are known) may be accomplished by repeated cycles of DNA polymerase reactions to generate

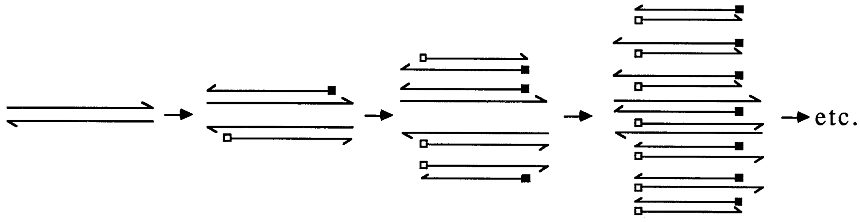

Figure 2-3. The polymerase chain reaction. A single copy gene is amplified using oligonucleotide primers (open and filled squares) and a DNA polymerase that synthesizes new, homologous strands. The DNA is denatured, primers rehybridized to the single-stranded DNA, and new strands synthesized again. After 30 rounds of amplification, the region of interest has been amplified 10^6–10^8-fold.

multiple copies of a particular DNA segment [26] (figure 2-3). Oligonucleotides homologous to DNA sequences at the 5′ and 3′ ends of the region of interest are used to prime the reaction. The amplified DNA may then be subjected to restriction digest to look for the presence or absence of a polymorphic restriction site, or hybridized to an allele-specific oligonucleotide probe.

Example 3: Deficiency of the lysosomal hydrolase glucocerebrosidase results in the most common of the sphingolipidoses, Gaucher disease [27, 28]. Three distinct phenotypic forms of the disorder are recognized. Type 1, or chronic nonneuronopathic Gaucher disease, is found with increased frequency among the Ashkenazi Jewish population. No ethnic predilection for types 2 (acute neuronopathic) or 3 (chronic neuronopathic) has been noted. Tsuji et al. [29] reported the nucleotide sequence analysis of a glucocerebrosidase mutation in the DNA from an Ashkenazi Jewish patient with type 1 Gaucher disease. In this patient, a single base mutation, in which an adenosine was changed to guanosine, was observed in exon 9. The authors used allele-specific oligonucleotide probes to demonstrate that this mutation was found exclusively in patients with the type 1 phenotype. However, genetic heterogeneity was found even among the Ashkenazi Jewish patients with type 1 disease; 15 of 24 such patients were heterozygous for this specific mutation, and only 3 of the 24 were homozygous.

Example 4: A different mutation, in which the leucine at residue 444 is changed to proline, is found frequently in types 2 and 3 Gaucher disease, and less frequently in patients with type 1 disease [30]. This mutation creates a new NciI restriction site and hence can be detected by Southern blot hybridization following NciI digestion of the DNA in question. The combination of allele-specific oligonucleotide probe hybridization (described in example 3) to detect the Asn-370-to-Ser mutation and restriction endonuclease analysis to detect the Leu-444-to-Pro alteration is informative for diagnosis and genetic counseling in 80% of patients with Gaucher disease [29].

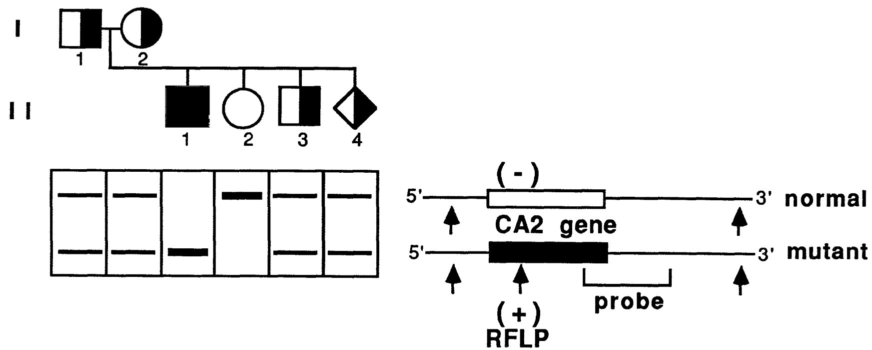

Figure 2-4. Hypothetical DNA diagnosis of autosomal recessive osteopetrosis by RFLP linkage, using the carbonic anhydrase II (CA2) gene. The precise mutation is unknown. Both parents are heterozygous for a polymorphic restriction site. The affected offspring (II-1) inherited both alleles with the polymorphic site (both (+) alleles), indicating that the (+) alleles from both parents carry the mutation. The fetus (II-4) is heterozygous for the RELP, and so will be a carrier of the disorder but not affected.

INDIRECT DETECTION OF MUTATIONS: LINKAGE ANALYSIS

Mutant gene known

Analysis of mutations in the beta-globin and factor VIII genes has demonstrated tremendous molecular heterogeneity among individuals affected by beta-thalassemia and hemophilia A [14, 18]. For this reason, the precise molecular defect in a particular family cannot be easily ascertained. The method of linkage analysis facilitates the molecular diagnosis of such disorders, in the absence of knowledge of the specific defect in a given family.

DNA polymorphisms (the presence or absence of restriction enzyme recognition sites) are used to differentiate the two alleles for a particular gene of interest. These polymorphisms are well known to exist in the normal population. They do not reflect the mutation at the locus per se, but merely serve to mark the allele carrying that mutation within the family. Such polymorphisms, when they lie within or flanking the gene of interest, can be used as markers for the mutant allele.

Example 5: An autosomal recessive form of osteopetrosis, associated with renal tubular acidosis and cerebral calcifications, results from a deficiency of the enzyme carbonic anhydrase II [31]. The gene encoding this enzyme is designated CA2 and maps to human chromosome 8 [32]. A polymorphic restriction site associated with the CA2 locus has been reported [33] and is of potential utility for prenatal diagnosis of this disorder (figure 2-4).

Mutant gene unknown—linkage analysis

For the vast majority of Mendelian phenotypes, the gene causing the disease is still unknown [1–4]. In some cases, linkage analysis using polymorphic

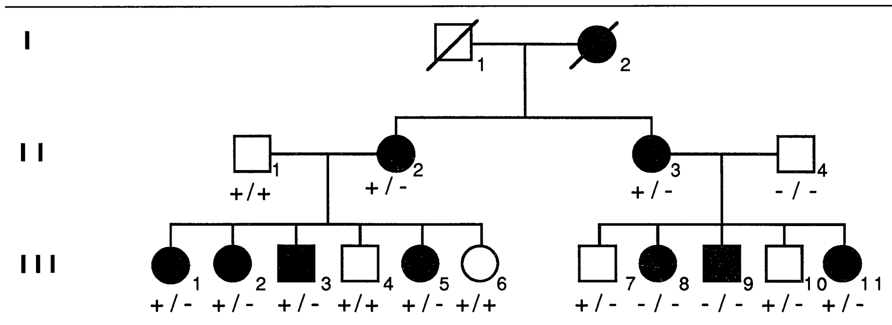

Figure 2-5. Three-generation pedigree with von Hippel–Lindau (VHL) syndrome, an autosomal dominant condition, analyzed using an RFLP associated with the *raf* oncongene on human chromosome 3. Affected sisters II-2 and II-3 are both heterozygous for the polymorphism. Each of their affected offspring inherited the maternal (−) allele, with the exception of II-11, who inherited her mother's (+) allele. Individual II-11 thus represents a recombinant event between the VHL locus and the *raf* polymorphic marker. The frequency of recombination in this family (1/11 opportunities for recombination) is consistent with the reported genetic distance of 11 centimorgans (cM) between the *raf* and VHL loci (1 cM = 1% recombination rate).

markers distributed throughout the genome has been successful in pinpointing the chromosomal location of the mutant gene. Close linkage between an anonymous DNA segment or randomly selected gene enables molecular diagnosis of genetic disorder, even in the absence of knowledge concerning the precise genetic locus causing the disease. In this approach, restriction fragment polymorphisms recognized by the anonymous marker serve to mark the mutant allele in a specific family. The predictive accuracy of the analysis depends on the genetic distance between the marker and the disease gene; the further the marker is from the gene, the greater will be the chances of meiotic recombination between the two loci and an inaccurate prediction of the disease status of the individual in question [9, 10].

Example 6: Von Hippel–Lindau (VHL) syndrome is an autosomal dominant disorder characterized by a predisposition to the development of malignancy, incluing renal-cell carcinoma, pheochromocytoma, pancreatic tumors, and hemangioblastomas of the central nervous system. Recent genetic linkage studies have shown that the VHL locus is closely linked, but not identical, to the *raf*1 oncogene on human chromosome 3p25 [24]. A maximum LOD score (that is, the greatest likelihood of linkage) was obtained at a recombination fraction of 0.11. This means, on average, that recombination will occur between the *raf*1 marker and the VHL locus in 11/100 meioses (figure 2-5); diagnosis based on the *raf*1 marker will be in error 11% of the time. This is an unacceptably high error rate for prenatal diagnosis; closer markers are needed before linkage studies can be preformed with a reasonable degree of accuracy.

Autosomal dominant polycystic kidney disease [6] and tuberous sclerosis [35, 36] are additional disorders causing significant renal pathology, for which gene localization has been accomplished but the gene identification has not.

FUTURE DIRECTIONS

Although tremendous strides in gene diagnosis have been made in the last decade, much remains to be done. Of the hereditary renal disorders, in many cases neither the mutant gene nor its chromosomal map location is yet known. For others, such as the autosomal dominant form of polycystic kidney disease, von Hippel–Lindau syndrome, and tuberous sclerosis, the chromosomal location is known but the mutant gene has yet to be identified. This process of so-called *reverse genetics* is best typified by the recent successes in identifying the gene causing Duchenne muscular dystrophy [37,38]. Moreover, for those disorders in which both the mutant gene and its map location are known (e.g., osteopetrosis with renal tubular accidosis and cerebral calcification), the precise nature of the mutations and their pathophysiologic consequences remain to be elucidated. As molecular genetic methods provide greater insight into the pathogenesis of the Mendelian disorders, we may begin to unravel the genetic component of common diseases with multifactorial etiologies.

CONCLUSIONS

The methods of diagnosis by DNA analysis have been presented and the advantages and disadvantages of each discussed. Direct detection of mutations is possible when the mutant locus is known and the mutation itself has been identified. Gross alterations of the mutant locus may be identified on Southern blots or by denaturing gradient gel eletrophoresis. Point mutations and more subtle alterations require allele-specific oligonucleotide hybridization or, if the mutation creates or removes a restriction recognition site, restriction endonuclease analysis. Indirect methods of gene diagnosis, by means of linkage studies using restriction fragment length polymorphisms, are used when the precise nature of the mutation is unknown; the mutant gene itself may or may not be identified. If the mutant locus is unknown, DNA fragments closely linked to the disease gene in question serve as markers for the mutant allele(s) in a family. Although a number of hereditary renal disorders can now be diagnosed by means of DNA analysis, completion of the list is sure to take us well into the twenty-first century.

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II. PRIMARY GLOMERULAR DISEASES

3. INHERITANCE OF GLOMERULAR DISEASES

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The wide spectrum of inherited glomerular diseases can be classified in four groups, as indicated in table 3-1. The genetic information available varies from one disease to another. In some conditions, such as Fabry's disease, the enzyme defect has long been identified, there are biochemical methods for early diagnosis, and DNA technology is being used to determine the molecular gene defect(s). In other conditions, such as Alport's syndrome and its variants, although clinical and genetic heterogeneity is well established, the antigenic defect of the glomerular basement membrane (GBM) has just been identified, and DNA analysis has been initiated only in the X-linked form. Lastly, in many cases such as primary familial glomerulonephritides, which are often sporadic and where familial aggregation has rarely been documented, genetic information is scarce. Yet these familial cases are of great interest because they illustrate how crucial genetic factors are in primary glomerular diseases. They also provide an opportunity to review the methodology that should be used to demonstrate genetic predisposition in polygenic diseases.

ALPORT'S SYNDROME AND VARIANTS

Alport's syndrome is an inherited disease characterized by progressive glomerular destruction and sensorineural hearing loss. In most cases, ultrastructural changes of the GBM are found, and GBM antigenicity is altered (see chapter 4). In affected males, the disease progresses to end-stage renal failure (ESRF), whereas its progression is slower or absent in affected or carrier females [1].

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Table 3-1. Inherited glomerular diseases*

1. Alport's syndrome and variants	
2. (Familial benign hematuria)	
3. Hereditary metabolic diseases with glomerular involvement	
Fabry's disease	
Lecithin-cholesterol acyltransferase deficiency	
Other identified or unidentified storage diseases	
Genetic amyloidosis	
(Diabetes mellitus)	
(Sickle-cell disease)	
(Complement defects)	
4. Familial glomerulonephritis	
Primary:	IgA nephropathy
	Minimal change disease, focal glomerulosclerosis, and related disorders
	Other types
Other forms:	Acute poststreptococcal glomerulonephritis
	Anti-GBM disease
	Hemolytic–uremic syndrome
	(Systemic lupus erythematosus)

*The diseases indicated in parentheses are not discussed in this review.

Unaffected obligate male carriers do exist in very rare instances ([2]; unpublished observations); this is relevant to genetic counseling.

In most families (83% of cases in our experience), inheritance of Alport's syndrome is compatible with X-linked dominant transmission [3]. In such a mode of transmission, all daughters of affected fathers should be affected. However, in the families studied by Hasstedt et al. [2], the corresponding percentage was 87%. Moreover, penetrance of microscopic hematuria in female heterozygotes was estimated to be 82% [2]. This explains the difficulty in detecting some of the asymptomatic carrier females. In X-linked dominant cases, no father-to-son transmission should be found, and affected women should have approximately 50% affected offspring [2].

Autosomal dominant inheritance, present in 12% of cases, is characterized by father-to-son transmission. It should be stressed, however, that in families in which affected men have a small number of children all of whom are females, the pattern of transmission may appear consistent with either X-linked or autosomal dominant inheritance. Lastly, in 5% of the kindreds, the mode of inheritance is compatible with autosomal recessive transmission, the disease being expressed in the offspring of consanguineous parents [3].

The extrarenal manifestations should also be taken into account when assessing the mode of inheritance. Hasstedt et al. [2] have stated that Alport's syndrome with macrothrombocytopenia is inherited as an autosomal dominant trait. However, in two familes, some male carriers in both kindreds presented with thrombocytopenia but without clinical renal involvement [4]. This pattern is quite unusual in classical Alport's syndrome, where affected males generally exhibit abnormal urinalysis. Definite information is lacking regarding transmission of Alport's syndrome associated with diffuse leiomyo-

matosis [4]. Likewise, data are scarce on the inheritance of *hereditary nephritis without nerve deafness* (i.e., hereditary renal disease, with predominant glomerular pathology, without metabolic or extrarenal abnormalities, progressing to renal failure in at least one member of the family). In our experience, based on seven families, inheritance was compatible with X-linked dominance in six and with autosomal dominant in one, a distribution close to that found in Alport's syndrome. In addition, ESRF was reached at similar ages in Alport's syndrome and in hereditary nephritis without deafness, 36.3 ± 2.1 years (n = 75) and 35.6 ± 7.1 years (n = 8), respectively.

Pedigree analysis provides valuable information regarding prognosis. In most families, the course of the disease follows a similar pattern over time [2, 3]. Hasstedt et al. [2] distinguished two subgroups of families. In the first group, called juvenile Alport's syndrome, mean age at which the patients reached end-stage renal failure was 18.9 ± 7.2 years, whereas it was 35.1 ± 6.7 years in the second group, or adult-type Alport's syndrome. It should be stressed that this observation applies only to males; the course differs greatly between affected women in various kindreds as well as among women in a given kindred [5].

An intensive search has been initiated to locate the defective gene of Alport's syndrome in the X-linked form, using recombinant DNA technology. These studies have revealed significant linkage between the Alport gene and marker loci (DXS 1, DXS 3, DXS 17, DXS 11, DXS 14, DXS 94, and DXS 101) in the middle of the long arm of the X chromosome [6–9], probably in the Xq 21 region. However, a more precise location of the mutant gene is necessary in order to develop an early diagnostic test. There is no information on the location of the mutant genes in other forms of Alport's syndrome. Preliminary data suggest that the linkage on the X-chromosome is similar in Alport's syndrome and in hereditary nephritis without deafness [9].

Genetic counseling should take into account the following points. 1) The mode of transmission in a given family is important. In X-linked dominant forms, affected females have a 50% risk of transmitting the disease to their offspring; sons of affected males are all healthy, whereas daughters are all affected. In autosomal dominant forms, there is a 50% risk of transmission to the offspring. 2) In all forms, the renal disease is more progressive in males than in females. 3) The age at which renal death occurs is similar among members of a kindred. In the coming years, DNA analysis should offer the possibility of prenatal diagnosis and of more precise counseling, at least in the juvenile form.

HEREDITARY METABOLIC DISEASES WITH GLOMERULAR INVOLVEMENT

Fabry's disease

Fabry's disease is an inborn error of glycosphingolipid metabolism due to the defective activity of the lysosomal enzyme, α-galactosidase A. This defect leads to the accumulation of neutral glycosphingolipids in body fluids and in the lysosomes of most tissues, particularly in the renal and cardiovascular

system. The disease is transmitted by an X-linked recessive gene [10]. In homozygous males, the first clinical manifestations, acroparesthesias, angiokeratoma, hypohydrosis, and corneal dystrophy, appear in childhood. End-stage renal disease develops in the fourth decade. Heterozygous females are usually asymptomatic, although some develop corneal opacities.

The diagnosis is based on the demonstration of deficient α-galactosidase A activity in plasma, leukocytes, or other tissues. This activity is nil in hemizygous males and intermediate in heterozygous females. However, due to random X-chromosomal inactivation, heterozygous subjects can express levels of enzyme activity from zero to normal values. In addition, with aging, inactivated X-chromosomal genes may become reactivated by demethylation. This explains the rising levels of α-galactosidase activity in older obligate heterozygotes [11]. Accurate detection of carriers is crucial from genetic counseling and antenatal diagnosis. Enzymatic techniques are tedious and may be inconclusive. DNA technology may provide more accurate identification of heterozygotes.

Originally, the Fabry gene was thought to be located on the short arm of the X-chromosome because of a linkage between Fabry disease and the X-linked blood group antigen Xga. Somatic cell hybridization studies have revealed that the α-galactosidase A gene is located on a small region of the long arm of the X-chromosome (Xq 22) [10]. Moreover, full-length cDNA encoding human-galactosidase A has become available. In families with gene rearrangements or an altered restriction endonuclease cleavage site, precise diagnosis can now be accomplished by Southern blot analysis. Unfortunately, this can be achieved in less than 5% of the affected families because deletions or insertions are uncommon or small or because point mutations are involved. In families with normal restriction patterns, restriction fragment length polymorphisms (RFLPs) have been identified in or flanking the α-galactosidase A gene [11, 12]. These probes were informative only in about 70% of the families investigated by Desnick et al. [11]. It may be expected that molecular diagnosis of Fabry's disease will become generally available in the near future.

Lecithin–cholesterol acyltransferase (LCAT) deficiency

LCAT, a serine protease containing 416 amino acid residues secreted by the liver, is the major cholesterol-esterifying enzyme in human plasma. Patients with LCAT deficiency develop lipid deposits in the cornea, hemolytic anemia, premature atherosclerosis, and glomerular lesions (foamy cells, increased mesangial matrix, and osmiophilic deposits in the GBM) that lead eventually to ESRF [13].

The mode of inheritance is autosomal recessive [14], and the molecular defect is heterogeneous: LCAT activity is virtually absent in homozygotes, wherease the LCAT protein may or may not be detectable [15]. Quantitation of LCAT activity allows identification of heterozygotes [14].

The LCAT gene has been located on the long arm of chromosome 16, near the haptoglobin gene. Human LCAT cDNA has been isolated and sequenced

]16, 17]. RFLP analysis using ten different restriction enzymes failed to reveal any difference between affected and normal subjects. The disease thus more probably results from point mutation rather than gene deletion or insertion [15, 16]. It would be of interest to determine whether the defect in the patients in whom circulating LCAT protein cannot be detected immunologically is caused by a deletion of all or of part of the LCAT structural gene.

Other storage glomerular diseases

Type I glycogen storage disease is characterized by deficient glucose-6-phosphatase activity. Family study supports an autosomal recessive form of inheritance. Renal disease was documented in 70% of the patients who were over ten years of age, and may progress to ESRF. The predominant histologic finding was focal segmental glomerulosclerosis [19].

Genetic forms of renal amyloidosis

The main characteristics of inherited forms of renal amyloidosis are summarized in table 3-2. Only familial Mediterranean fever (FMF) is transmitted as an autosomal recessive trait. Environmental factors and/or additional genetic predisposition account for the greater prevalence of amyloidosis complicating FMF among Sephardic Jews than among Ashkenazi Jews and Armenians [22]. Such genetic predisposition has also been demonstrated in reactive AA amyloidosis complicating juvenile arthritis [25].

HEREDITARY DISEASES WITH GLOMERULAR AND EXTRARENAL INVOLVEMENT

Hereditary osteo-onychodysplasia or nail-patella syndrome (NPS) is a connective tissue disease characterized by nail dysplasia, multiple bone changes (absent or hypoplastic patellae, iliac horns, and elbow abnormalities) and, in 30% to 55% of the cases, kidney involvement. The glomerular basement membrane is thickened, and contains bundles of cross-striated collagen fibrils (see review in [26]). The mode of inheritance of NPS is autosomal dominant. However, GBM abnormalities similar to those seen in NPS have been observed in three families without bone or nail changes [30, 31]. In one of these families autosomal recessive inheritance was suggested [31]. These cases may represent a partial expression of the NPS mutant gene or an independent genetic form of GBM disease. The NPS locus is closely linked to the ABO blood group [27] and the adenylate kinase loci [28]. The NPS gene has been mapped by family linkage studies [29] at the distal extremity of the long arm of chromosome 9 (9q 34).

Various unspecific glomerular changes may be found in various inherited diseases, such as in the Drash syndrome (see below), the Bardet-Biedl syndrome, or in hereditary multiple osteolysis. Renal involvement has been claimed to be the sixth cardinal sign of the Bardet–Biedl syndrome in addition to obesity, mental retardation, polydactyly, pigmentary retinopathy, and

Table 3-2. Genetic renal amyloidosis

Clinical type		Mode of inheritance	Clinical features (apart from renal involvement)	Chemical type
"Ostertag" (without neuropathy)	[19]	Autosomal dominant	Hepatosplenomegaly	Unknown
Type I familial amyloid polyneuropathy (Swedish type and others)	[20]	Autosomal dominant	Peripheral neuropathy followed by systemic involvement	AF*
Familial Mediterranean fever		Autosomal recessive + additional predisposition for amyloidosis	Recurrent and colchicine-sensitive attacks of fever, polyserositis, and arthritis	AA**
Muckle–Wells syndrome	[22]	Autosomal dominant	Nerve deafness, urticaria, limb pain	Probably AA** [23]
Recurrent febrile illness with amyloidosis	[23]	Autosomal (?) dominant	Recurrent colchicine-unresponsive attacks of abdominal pain or arthritis	Unknown

*AF protein derives from variants of pre-albumin (or transthyretin).
**AA protein derives from serum amyloid A.

hypogenitalism. The mode of transmission is autosomal recessive [32]. Urinary tract abnormalities such as caliceal changes and occasionally vesicoureteric reflux are found. Glomerulosclerosis and mesangial proliferation, in association with cystic and tubulointerstitial changes, are demonstrated by renal biopsy [32, 33]. It has been suggested recently that GBM abnormalities may also develop during the early course of the disease [34].

Hereditary multiple osteolysis is transmitted as an autosomal dominant trait. Progressive disappearance of carpal and tarsal bone structure leads to painful swelling of wrists and feet during childhood. Renal involvement occurs later in life and may progress to ESRF. Renal biopsy shows unspecific arterial sclerosis and glomerulosclerosis [35, 37].

Small-vessel hyalinosis involving the digestive tract, retina, and the kidney (with glomerular mesangiolysis), associated with phenotypic abnormalities (such as poikiloderma and early hair-greying) has been observed in one family in which transmission was compatible with autosomal recessive inheritance [38].

FAMILIAL GLOMERULAR DISEASES: PRIMARY AND OTHER FORMS

It is generally assumed that glomerular diseases have a random distribution in the population. In most series, there is no mention of affected relatives. There is evidence, however, that points towards a genetic determinism in glomerulonephritis (GN). This includes cases of biopsy-proved familial GN, ethnic differences in the prevalence of some glomerular diseases, and similar immune abnormalities in relatives or association of certain forms of GN with histocompatibility (HLA) antigens (table 3-3).

It must be emphasized that multiple cases of the same disease in a family

Table 3-3. Epidemiologic and genetic characteristics of primary glomerular diseases

Disease	Incidence * [ref]	Sex and age preponderance	Affected populations	Familial cases	Association with HLA [ref]
MN	Commonest cause of NS in adults – Idiopathic MN in France = 0.75 [40] – Secondary MN in France = 0.75 [40] – MN in the Netherlands = 0.9 [41]	Male; adults	All populations	Extremely rare (twins, sibs)	– Series of Caucasian patients DR3 in 67%–75% patients vs 20%–23% in controls; RR = 4–12 [51] B8, B18, BfF1: contradictory results – Series of Japanese patients DR2 in 74%–80% patients vs 36%–39% controls; RR = 6–7 [53]
IgA N	Most frequent biopsied glomerular disease in adults – In France = 3.0 [40] – In the Netherlands = 1.9 [41]	Males; 20–30 yrs old	Caucasian and Asian; exceptional in Blacks	Not rare (31 families in France) – Most often sibs – Also successive generations Multiple patients in related pedigrees in Kentucky	– Two series of Caucasian patients (France) DR4 in 43%–49% patients vs 13%–20% controls; RR = 3.9 [61] No confirmation in series from Europe, North America – Series of Japanese patients DR4 in 58%–66% patients vs 34%–41% controls; RR = 2.7–5.1 [53]
Type I MPGN	Recent decrease in European countries – In France = 0.25 [40]	Young adults; both sexes	Rare in Blacks	Rare – Few pairs of sibs – Parent–sibs in one family – Only males in one family	– One series of Caucasian patients (U.S.A.) Haplotype B8, DR3, C4AQ0 in 26% patients vs 1% controls [65]
Type II MPGN	Exceptional Not known	Not known	Not known	None	– One series of Caucasian patients (U.K.) DR7 in 8/11 patients with Nef; RR = 9.9 [71]
FNS	Frequent – Children in the U.K. and the U.S. = 1–2 [42] – Adults in France MCNS = 0.35; FSGS = 0.5 [40] – Adults in the Netherlands MCNS = 0.6; FSGS = 0.9 [41]	Children; males	All populations	Not rare More frequent in FSGS – Sibs most often – Also successive generations	– Series of Caucasian (France, Australia, Spain) children with CSNS DR7 in 54%–75% patients vs 18%–38% controls; RR = 4.5–6.9 [77] – One series of Caucasian (France) children with CSNS DR3/DR7 in 30% patients vs 4% controls; RR = 9.3 [77]

Table 3-3. (cont.)

Disease	Incidence * [ref]	Sex and age preponderance	Affected populations	Familial cases	Association with HLA [ref]
					– One series of Caucasian (France) adults: no association – Two series of Japanese patients DR8 and DQW3 in one; DQW53 in another [15]
APSGN	Relatively frequent; sporadic cases; endemic in some countries with epidemic outbreaks Recent decrease in Europe and in the U.S. – In France = 0.05 [40]	Children	All populations	Frequent – Sibs nearly always	– One series of Caucasian patients (Venezuela) DR4 in 50% patients vs 21% controls (RR = 3.8) [84]
Anti-GBM disease	Extremely rare – In the U.K. = 20 patients/yr [43]	Men 20–30 yrs old Women 50–60 yrs old	Caucasian; unknown in Blacks	Extremely rare – Twins, sibs, cousins	– Series of Caucasian patients (U.K., Australia) In the U.K.: DR2 in 89% patients vs 19% controls; RR = 36 [85] More severe nephritis in B7-DR2 pts
HUS	Main cause of acute rental failure in infancy and childhood; sporadic cases; endemic in some countries with epidemic outbreaks – Children in the U.K. = 0.25–0.36 [44] – Children in the U.S. = 1.16 [45]	Less than 3 yrs old	All populations	Not rare – sibs most often – Successive generations	Not studied

*Incidence is expressed as the number of patients per 100,000 inhabitants per year. MN: membranous nephropathy; IgA N: IgA nephropathy; MPGN: membranoproliferative glomerulonephritis; FNS: familial nephrotic syndrome; APSGN; acute poststreptococcal glomerulonephritis; HUS: hemolytic uremic syndrome; MCNS: Nephrotic syndrome with minimal change; FSGS: focal segmental sclerosis; glomerular; CSNS: corticosteroid-sensitive nephrotic syndrome; RR: relative risk; Nef = nephrotic factor.

may simply occur by chance, or the cases may be due to exposure to the same environmental factors [39]. It is generally agreed that a disease aggregates in families when the incidence or the prevalence of the disease is higher among the relatives of affected individuals than it is in the general population from which they were selected [39]. Unfortunately, the prevalence of any glomerular disease in a given population is rarely known [40–45]. Another method of assessing for familial aggregation is to compare the incidence of the disease among relatives of patients to that found among relatives of a control group. To our knowledge, this type of study has not been performed in glomerular diseases. Once familial aggregation is established, the second step is to try to separate genetic from environmental effects [46]. Some of the methods available for this purpose (twin studies, adoption studies, etc.) have never been applied to glomerular diseases; others, such as the search for an association between a disease and a genetic system, have been widely used. The most commonly mentioned genetic system is the major histocompatibility, or HLA locus (class I and II genes located on chromosome 6). The basic approach consists of determining the HLA phenotypes in a group of unrelated patients with a given disease and comparing this group with a group of unrelated individuals of the same ethnic origin. The relative risk (RR) is a measure of the likelihood for an individual to develop the disease conferred by a given genetic marker. Three mechanisms may explain the association of an HLA antigen with a certain disease in a population [47, 48]. An association can be observed if the frequency of a disease and of an HLA antigen are higher in a subgroup than in the rest of the population. Such a stratification effect may induce an apparent association, even though no causal link exists between HLA and the disease. Linkage studies and the *sib pair method* (see following) are approaches that help to rule out this stratification effect. A second possibility is that a particular HLA antigen confers susceptibility to the development of a certain disease, although most people who have the antigen remain free of the disease. The susceptibility may be related to a subtype of the HLA antigen that could be identified by RFLP analysis of HLA genes. Finally, the association of a particular antigen with a disease may be caused by the existence of susceptibility gene(s) in or near the HLA region in linkage disequilibrium with the HLA locus. There may also be non-HLA genetic systems, for example the Gm system (genes encoding the immunoglobulin heavy chain allotypes on chromosome 14) and complement components that affect the familial incidence of certain glomerular diseases.

There are different potential mechanisms whereby disease-associated genes could play a role. They may determine the immune response to environmental or autologous antigens (i.e., act as immune-response genes), but they may also control the synthesis of essential components involved in the response to infectious agents. They may also participate in the endogenous removal of immune complexes. Lastly, certain genes may encode cell receptors of exogenous (viral) agents.

Many diseases do not appear to be inherited as simple dominant or recessive Mendelian traits. These diseases can be regarded as multifactorial, meaning that a number of different environmental and genetic factors contribute to the susceptibility of the individual. Until 1970, all genetic influences were regarded as polygenic, implying that many genes have a small but cumulative effect on susceptibility. Polygenic influences would lead to a continuum of genetic predisposition to a given disease. According to this model, a disease becomes manifest when the person's underlying predisposition passes a *threshold value*. It was later suggested that a genetic predisposition could be determined not only by polygenic inheritance and environmental factors, but also by a gene having a major influence on susceptibility (mixed model) [49].

An effective means of verifying a model (polygenic model, mixed model) of genetic transmission of disease susceptibility is through linkage analysis (i.e., examination of the distribution of a disease and of a marker among members of randomly sampled families.) Difficulties arise from incomplete penetrance, varying age-at-onset, and variability of symptoms, but particularly from the lack of a histologic diagnosis, which can be obtained only by renal biopsy. The study of a marker in affected siblings from different families (sib pair method) overcomes this difficulty. Affected siblings are examined for the number of shared marked alleles. In the absence of linkage between a disease susceptibility locus and a marker locus, the expected proportions of sibling pairs with 0, 1, and 2 shared marker alleles are ¼, ½, and ¼, respectively. By contrast, if there is a linkage, affected pairs of siblings who are concordant for the disease are expected to be more alike for the marker than pairs who are discordant for that particular condition [48].

Primary glomerulonephritis

Membranous nephropathy (MN)

MN is the most common form of nephrotic syndrome (NS) in adult patients. Familial occurrence is extremely rare (one pair of monozygotic twins, five pairs of brothers) [50]. An increased frequency of HLA-DR3 among these patients was first reported in the United Kingdom and confirmed in other countries of Europe. Studies in Japan have revealed a highly significant correlation between MN and DR2 [53]. An increase in B18 antigens and in BfF1 was noted in the United Kingdom and was interpreted to reflect a poor prognosis. This finding was not confirmed in other countries, where instead a significant increase of HLA-B8 appeared to be associated with a poor outcome [51]. However, the response to therapy does not seem to be affected by the presence of HLA-DR3/B8 antigens [52]. An association of HLA-DR3 has also been demonstrated with MN secondary to gold or penicillamine therapy.

The association between MN and HLA-DR3 supports the assumption that MN is due to an autoimmune process. Diseases due to disturbances in autoimmunity (diabetes, thyroiditis, SLE, celiac disease) are frequently associated with HLA-DR3. Furthermore, several studies using RFLP analysis have revealed that rare HLA class II variants could be observed in certain autoimmune

diseases. Sacks et al. [54] investigated molecular polymorphism of HLA class II beta chains in the haplotype HLA-DR3 to determine whether patients with idiopathic MN express the normal HLA-DR3-associated polymorphisms or reveal class II variants. Only normal patterns were identified.

Results concerning the frequency of Gm allotypes in population studies have been contradictory. Using RFLP analysis, Demaine et al. [55] suggested that immunoglobulin heavy chain switch region polymorphisms and T-cell receptor constant beta chains may be important in the pathogenesis of MN.

IgA nephropathy

IgA nephropathy is the most common primary glomerulonephritis in Caucasian and Asian populations. Since the original description of primary IgA nephropathy, IgA deposits have been described in other diseases such as Henoch-Schönlein purpura (HSP) and liver diseases. The possibility of differences between Caucasian and African patients in the frequencies of RFLPs between the loci encoding the constant regions of the IgA1 and IgA2 heavy chains (chromosome 14) is under investigation [56].

Studies of large populations of patients with IgA nephropathy have provided anecdotal evidence of familial occurrence. Furthermore, apparently healthy relatives of patients with IgA nephropathy were found to have a high frequency of immune abnormalities (increased serum IgA levels, increased amount of IgA-bearing cells, etc.), suggesting a genetic predisposition [57]. Following the first report of two brothers with biopsy-confirmed IgA nephropathy, multiple instances of familial IgA (22 families) or of both IgA nephropathy and HSP (4 families) have been identified [58]. A cooperative retrospective study conducted in France revealed 31 families in which two (or more) members had biopsy-confirmed IgA nephropathy. Ten additional families had both IgA nephropathy and HSP. Affected members were either parent–offspring or more often siblings. The long interval frequently observed between the apparent onset of the disease in various family members favours the existence of genetic abnormalities rather than of environmental factors. The two main characteristics of these patients are the young age at the apparent onset of the disease and the severity of the nephropathy. These data suggest differences between familial and sporadic cases, which occur later on in life and are more benign. Egido et al. [57] have also identified immunologic differences between these two categories of patients. Specifically, there was a significantly increased activity of IgA helper T-cells in patients belonging to familial IgA nephropathy as compared to those with the sporadic form of the disease.

The discovery of 14 patients with IgA nephropathy in potentially related pedigrees from Kentucky (U.S.A.) provides an additional argument in favor of a genetic predisposition. Sixty percent of the patients were related to at least one other patient in the region [59]. The authors suggested that the gene(s) producing susceptibility of IgA nephropathy might have been carried into the eastern part of Kentucky by one or several of the original settlers. Pedigree segregation analysis indicated the existence of a recessive allele for high plasma

IgA concentration, which might be a minor (rather than a major) etiologic factor in IgA nephropathy [60].

Studies of Gm and Km allotypes in IgA nephropathy showed inconsistent results. Preliminary results of analysis of RFLPs of the DNA encoding the IgA heavy chains have not revealed at present any modification of the gene [56]. Hereditary abnormalities of the complement components (C4, H, P) have been observed rarely. An increased frequency of null C4A and C4B phenotypes and of C3F alleles has been reported in patients in the U.S.A. [61].

The association of HLA antigens with IgA nephropathy has been widely studied. Discrepant results concerning the frequencies of HLA-B35 or of HLA-B12 have been reported. An association with HLA-DR4 has been documented in all studies from Japan and in two studies from France. The results concerning the relationship between prognosis and HLA-DR4 reported from Japan are controversial. A number of studies done in Europe and in North America failed to confirm an association betwen HLA-DR4 and IgA nephropathy [61]. Interestingly, a study of HLA haplotypes of affected siblings from different families revealed an excessive number of HLA-identical siblings [58]. Further systematic studies of affected sib pairs are needed before it can be concluded that there is a linkage between HLA and IgA nephropathy.

Membranoproliferative glomerulonephritis (MPGN)

Type I (with subendothelial deposits) and type III MPGN (with both subendothelial and subepithelial deposits) probably represent variants of the same disease rather than separate entities.

Berry et al. [62] were the first to report multiple cases of MPGN in two sibships. Only eight additional families with multiple affected siblings have been reported. Interestingly, in one sibship, one brother had type I and the sister had type III MPGN. In one family the disease (lobular GN) occurred in four brothers and the father, and in another the disease occurred only in males of two successive generations [63, 64].

An increased frequency of haplotype HLA-B8, C2C, BfS, C4AQ0B1, HLA-DR3 was noted in one series of patients in the U.S.A. with MPGN type I and type III. Patients having this extended haplotype had poorer prognosis than those without the haplotype. Welch et al. [67] suggested that the disease-susceptibility genes could reside in this specific extended haplotype and that other genetic or environmental factors act in concert to express the disease.

Additional support for genetic predisposition comes from the description of patients with hereditary C1Q, C2 (homozygous and heterozygous), C3 (homozygous and heterozygous), C6, combined C6–C7, and H deficiencies, some of whom either had type I MPGN or possibly had related ultrastructural changes. Patients with abnormal C3 and C7 also presented with MPGN. In most families only one member developed MPGN. However, H deficiency has been noted in two brothers who both had an atypical form of MPGN [66] and an abnormal C3; renal involvement was also observed in a pregnant

woman and her infant [67]. Two patients with MPGN and CR1 deficiency have recently been described [68] (see chapter 18).

The association of liver disease, subepidermal immunoproteins, and type I MPGN was reported in four siblings. The occurrence of GN has been described in a few patients deficient in alpha I-antitrypsin (alpha I-antitrypsin is coded by a pair of codominant alleles on chromosome 14). Most (but not all) cases have been type I MPGN. All patients had cirrhosis of the liver [69]. There was no report of familial cases of MPGN in these kindreds.

MPGN type II, or dense-deposit disease, is considerably less common than type I. The persistent C3 activation observed in these patients is due to a specific autoantibody, the so-called nephritic factor (NeF). NeF is also found in patients with partial lipodystrophy, who appear to be prone to glomerulonephritis, usually MPGN type II. To our knowledge, there is no reported familial case of MPGN type II. Predisposition to the generation of NeF was suggested by the presence of partial C3 deficiency associated with hypomorphism of a C3 variant in the relatives of a patient with MPGN [70]. The finding of increased HLA-DR7 in one small series of patients with NeF needs confirmation [71].

Nephrotic syndrome with minimal changes (MCNS), focal segmental sclerosis (FSG), and diffuse mesangial sclerosis (DMS)

The occurrence of NS in siblings was first described by Fanconi in 1951, while two different studies have indicated a familial aggregation. In a European survey performed in 1969 that excluded Finnish cases, it was found that 63 of 1877 nephrotic children had family members affected by the same disease [72]. The 3.35% incidence was higher than that of NS in the general population. Most families had two or three affected sibs. Two successive generations were rarely affected. Studies of 15 pairs of these affected sibs showed a high degree of concordance concerning age at onset, renal morphology, and outcome [73]. Those with onset in infancy had poor prognosis. However, all subsequent reports did not confirm this later finding.

Bader et al. [74] were the first to investigate carefully the mode of inheritance in familial NS. Pedigree information was obtained from 70 consecutive patients referred to a pediatric clinic in the U.S.A. from 1968 to 1971. The investigators found one pair of monozygotic twins, five sibpairs, and two first cousins from consanguineous families who had NS. The recurrence risk in sibs of affected patients (6%) was higher than the incidence in the general population. The authors concluded that the family data were more compatible with a polygenic determination of the trait, rather than with a simple recessive mode of inheritance. Few subsequent reports dealt with corticosteroid-sensitive MCNS. In one family there were two sibs affected at the ages of 3 and 7 weeks respectively, and in another family, subjects from two successive generations were affected during childhood.

By contrast, there are an increasing number of reports of FSG in siblings.

Tejani et al. [75] found three families with two or more affected members among 112 patients and identified six other families in the literature; an additional six families with affected sibs have subsequently been reported [76]. Familial FSG occurring in successive generations has also been described in patients presenting with or without NS. Lastly, the occurrence of familial NS with IgM deposits has been reported.

Another indication of possible genetic predisposition is the reported association between MCNS and HLA, mostly in children. First reports from the United Kingdom showed an increased frequency of HLA-B12. Subsequently, reports from different countries showed an increased frequency of HLA-DR7 in children with corticoid-sensitive idiopathic NS. The absence of an increased frequency of HLA-DR7 in adult patients with MCNS suggests that the disease differs from MCNS in children. Some investigators have linked HLA type to atopy, but not all studies have confirmed this relatioship. An association of corticosteroid-resistant NS with DR3 was recently demonstrated in France [77]. Furthermore, a high relative risk (RR) was associated with the heterozygous phenotype DR3/DR7. This phenotype correlated with an early onset of the disease and with lesions of FGS. These data suggest that the corticoid-sensitive and the corticoid-resistant forms of NS have different immunogenetic components.

Peculiar associations have been described: MCNS, nerve deafness, and hypoparathyroidism in two male siblings; MCNS, Friedreich's ataxia, and convulsive disorders in two male siblings; NS with FSG, congenital microcephaly, and hiatus hernia in two siblings from two families; and NS with FSG, hydrocephalus, thin skin, and blue sclerae in two brothers. Further family studies are needed before suggesting that these associated abnormalities are hereditary.

Congenital nephrotic syndrome of the Finnish type, a well-known autosomal recessive disease, is beyond the scope of this chapter (see chapter 7). Diffuse mesangial sclerosis (DMS) is an extremely rare disease, and its differentiation from Finnish type NS may be difficult. The possibility of hereditary forms was mentioned in the first review of the disease by Habib and Bois [78], who described two families. Eight other families are known [79]. Identical twins and two or more sibs were affected. The familial occurrence suggests a hereditary (autosomal recessive?) disease. This, however, remains to be demonstrated by genetic analysis.

A similar pattern of glomerular involvement is observed in the Drash syndrome characterized by the association of Wilms' tumor, pseudohermaphroditism, and nephropathy. Habib et al. [80] proposed extending the concept of Drash syndrome to all patients who, in association with the distinct glomerular pattern, had either Wilms' tumor or male pseudohermaphroditism. The possibility of a familial congruence of the Drash syndrome is supported by the description of two families (two pairs of twins with male pseudohermaphroditism and two sibs with Wilms' tumor and glomerular lesions). The gene responsible for Wilms' tumor has been assigned to chromosome 11. The location of the gene possibly responsible for Drash syndrome is not known.

Miscellaneous glomerular diseases

Unusual glomerular changes have been reported in some families: diffuse round mesangial deposits of C3 in one [81], and giant fibrillar deposits in another [82].

Other forms of glomerulonephritis

Acute poststreptococcal glomerulonephritis (APSGN)

The occurrence of familial cases of APSGN has long been recognized. The disease can reach epidemic proportions in closed communities. The spread of the disease among relatives is mainly attributed to overcrowding and adverse socioeconomic factors, although a genetic predisposition cannot be ruled out. Interestingly, familial occurrence of GN has also been described after varicella and typhoid fever.

Familial studies were performed in several countries with high prevalence of APSGN. In Colombia, a study of 311 sibs from 65 families revealed that 15% of the subjects (excluding index cases) had subclinical or clinical GN [83]. A prospective study performed in families of 22 patients with sporadic APSGN from Venezuela revealed that 73 out of 77 siblings and 33 of the parents developed streptococcal infection. Fourteen sibs had nephritis (symptomatic in 3 and symptomatic in 11) and only one parent had subclinical GN. The 37.8% attack rate in siblings at risk was higher than the attack rate in the general population during epidemics. The proportion of affected sibs, of approximately 25%, corresponds to the inheritance of a Mendelian recessive trait [84]. However, there is no genetic support for this assumption.

Few reports on the association of APSGN and HLA antigens have been published. An increased frequency of HLA-DR4 has been observed in one population [84].

Antiglomerular basement membrane antibody-mediated disease (anti-GBM disease)

Only a few familial cases (two sets of identical twins, three pairs of siblings, two pairs of cousins) have been reported. In a series of 41 patients known to have siblings, two siblings have developed anti-GBM disease (Rees, Lockwood, and Peters, unpublished).

A strong association between anti-GBM disease and HLA antigens was first noted in the United Kingdom when HLA-DR2 was reported in nearly 90% of patients. HLA-DR2 was found with the same high frequency in patients with or without pulmonary hemorrhage and with more or less severe renal insufficiency. However, patients who inherited HLA-B7 together with HLA-DR2 had more severe nephritis [85]. Further studies of the same group showed that inheritance of a specific Gm (Gm 1, 2, 21) allotype was strongly associated with the disease and that patients heterozygous at the Gm complex had higher titers of autoantibodies than did homozygotes [86]. Specific RFLPs detected by DNA encoding of the switch region for the heavy chain have been associated with anti-GBM disease [87].

Hemolytic–uremic syndrome (HUS)

HUS has long been recognized among the main causes of acute renal failure in infancy and childhood. The disease may also appear in adults. The renal involvement includes both arteriolar and glomerular lesions. Many triggering mechanisms are known, such as infections in children, and oral contraceptive treatment or pregnancy in adults. A report of two adopted nonrelated siblings and another report of husband and wife having the disease at about the same time give support to the role of common environmental factors.

The families of 13 children with HUS were investigated in a prospective study performed in California (U.S.A.) [88]. No signs of the disease were observed in the parents. Of the 13 siblings, five had diarrhea and three of these had HUS. In 1975, Kaplan et al. [89] collected reports of 83 siblings with the disease in 41 families located in various parts of the world. The patients were divided into two groups. Group 1 comprised 27 families, 25 of them coming from areas in which HUS is endemic. The disease occurred in 54 members of these families, with onset less than one month apart. The progress was relatively good (19% mortality). Group 2, included 14 families and 29 cases, with onset more than one year apart. Eleven of these families came from nonendemic areas. Four of the patients in this group had recurrences, whereas no recurrences were reported in group 1. Nineteen of the patients (68%) died. There were seven sets of twins, five of them from endemic areas. The authors suggested that HUS in group 1 could be the result of an environmental factor (or factors) and that patients in group 2 had a genetic predisposition to the disease. Subsequently, there have been other reports of affected sibs (7 families). Patients usually had the onset of the disease at the same age (as children or as adults), although in some families both children and adults were affected [90]. However, the prognosis did not conform to the pattern described by Kaplan et al.

Of note is the HUS that occurred in two or three successive generations. Most often, patients were adults and the prognosis was poor. It was not unusual in these families to find additional relatives who died without being examined but whose clinical symptoms were highly suggestive of HUS. The sex distribution was equal, although in one family all four affected patients were female and in another all were males [90].

Thrombotic thrombocytopenic purpura (TTP) shares some features with HUS and may be a more generalized manifestation of the same pathological process. The occurrence of TTP in a mother and of HUS in her daughter suggests that they could represent different expressions of the same disease [91]. TTP has been described in siblings and in successive generations [92].

There are conflicting hypotheses about the pathogenesis of HUS. Recent studies have led to the suggestion of an inherited or acquired disturbance in prostacyclin metabolism. Prostacyclin stimulation was found to be consistently low in one woman and in her two apprarently healthy sons. A disturbance of

prostacyclin metabolism was also found in children with HUS and in 18 of their 42 first-degree relatives. This is the first piece of evidence that some patients may have a genetic predisposition towards HUS. However, Pirson et al. [90] were unable to demonstrate a deficiency of prostacyclin-regulating factors in three patients and their 12 first-degree relatives.

To our knowledge, there is no study of HLA antigens in this population. In a few families, both HLA identical and non-HLA identical affected sibs were reported. The role of complement activation in HUS is not clear. Persistent hypocomplementemia was found in three related patients with HUS as well as in several healthy members of the family. Recurrent HUS has been associated with a rare allele of C3 [93].

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4. IMMUNOGENETICS OF THE GLOMERULAR BASEMENT MEMBRANE

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The nature of the genetic defect responsible for the abnormalities of kidney, cochlea, and eye that are characteristic of Alport's syndrome has been a matter of interest to nephrologists, geneticists, and pathologists for decades. In recent years this entity has been the subject of intensive laboratory research, the results of which indicate that the primary defect involves the collagenous components of basement membranes. In this chapter we will discuss the insights that research has provided, as well as the fundamental questions that remain to be addressed.

THE PRIMARY RENAL DEFECT IN ALPORT'S SYNDROME LIES IN THE GLOMERULAR BASEMENT MEMBRANE (GBM)

Any history of the modern era of Alport's syndrome research must begin with a description of the series of ultrastructural studies that in the late 1960s and early 1970s first focused attention on the GBM as the site of the primary renal defect in the disorder. Up until that time, the Alport's nephropathy was thought to be first and foremost a tubulointerstitial process, highlighted by the presence of foam cells in the interstitium. Then investigators in several laboratories, using electron microscopy, observed unique alterations of the GBM in Alport's patients: the GBM was markedly and diffusely thickened, and the normally homogeneous lamina densa was fragmented into multiple, interlacing strands in a basket-weave pattern [1–8]. Electron-dense granules 20–90 nanometers in diameter were often seen between strands of lamina densa.

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While focal lamina densa splitting may be present in a variety of glomerulonephritides [9], the diffuse, basket-weave transformation of the lamina densa is now accepted as a finding pathognomonic of Alport's syndrome [10–13].

Subsequent studies revealed that the GBM was abnormally thin, secondary to thinning of the lamina densa, early in the course of the disease in affected males [14]. The degree and extent of lamina densa fragmentation were found to correlate with age in affected boys, and also with the severity of proteinuria [15–17]. On the other hand, GBM thinning accompanied by focal lamina densa thickening and splitting has been found to be the predominant abnormality in affected females throughout life [17, 18], although diffuse GBM thickening and lamina densa splitting may develop as well [19].

Ultrastructural studies of organs other than the kidney in Alport's syndrome have been few. Although Martinez-Hernandez and Amenta described lamellation of the epidermal basement membrane and dermal vascular basement membranes in members of an Alport's kindred [20], we and other investigators have been unable to confirm this finding [21]. Recent studies of the anterior lens capsule (ALC) in patients with anterior lenticonus, another lesion pathognomonic of Alport's syndrome [22], are of great interest. It has been known for years, by light microscopy, that the ALC is markedly thinned in the region of lenticonus [23]. The recent electron microscopic studies of Streeten et al. [24] showed not only thinning but also disruption of the ALC at several sites in a woman with anterior lenticonus. Since the ALC is a typical basement membrane [25], anterior lenticonus, like the glomerulopathy of Alport's syndrome, appears to be related to a basic defect in basement membranes [26].

THE MISSING ANTIGEN HYPOTHESIS

An important step forward was taken in 1980 with the demonstration by Olson and colleagues [27] that autoantibodies to GBM in sera from patients with Goodpasture syndrome failed to bind to GBM in tissue sections from patients with Alport's syndrome, although these antibodies bound normally to GBM of patients with other forms of glomerulonephritis. This finding was quickly confirmed by investigators in other laboratories [28], although other Alport's patients were reported whose GBM retained the capacity to bind Goodpasture antibodies [29, 30]. It is of interest that most, though not all, of these latter patients have been female. Recently it was shown that the GBM of some male patients with Alport's syndrome lacked reactivity with a monoclonal antibody directed against the Goodpasture antigen [31]. Since Goodpasture antibodies also showed no reactivity in vitro with the GBM of the fetus and infant [32], it was postulated that GBM changes in Alport's syndrome reflect the persistence of an immature GBM, indicated by the failure of the Alport's GBM to acquire a normal antigen [20]. Researchers in our laboratory, however, have demonstrated that the Goodpasture antigen is hidden, rather than absent, in fetal and infant GBM by denaturation of tissue sections

in acid-urea prior to immunostaining [33–35], while the same techniques fail to uncover the Goodpasture antigen in Alport's GBM [36].

In 1982, McCoy and Wilson [37] described a male with Alport's syndrome who had developed anti-GBM antibody-mediated glomerulonephritis following renal transplantation. Interestingly, antibodies in this patient's serum reacted with the GBM of his renal allograft as well as with all normal GBM, but showed no reactivity with GBM of his native kidneys or with GBM in kidney tissues from six other male Alport's patients. Further, the reactivity of this patient's serum with normal GBM was not blocked by preabsorption with Alport's kidney but was completely abolished by preabsorption with normal kidney. McCoy and Wilson hypothesized that the Alport's GBM lacked a normal GBM antigen, and that in their patient transplantation of a normal kidney had elicited an immune response to that antigen.

Since this report, several other Alport's patients with posttransplant anti-GBM nephritis have been described [36, 38–40]. To date, each of these patients has been male. Querin et al. [40] found linear deposition of IgG along the GBM of allografted kidneys in 5 of 34 (14%) patients with Alport's syndrome. These patients were also males, without exception. One of these patients had histological evidence of glomerulonephritis. Clinically significant anti-GBM nephritis occurs so rarely in transplanted Alport's patients (probably less than 1%) that it is unnecessary to restrict access to transplantation, including the use of kidneys obtained from living donors. However, patients who develop anti-GBM nephritis in a first allograft would seem to be at high risk for recurrence in subsequent transplants [38, personal observations].

Rees et al. [41] found an increased susceptibility to the development of anti-GBM antibodies in individuals who were HLA-DR2-positive, while HLA-B7 was associated with greater severity of anti-GBM nephritis in HLA-DR2-positive patients. Available data do not suggest such correlations in Alport's patients with allograft anti-GBM nephritis, though the number of cases is small [38, 40, personal observations]. Why so few Alport's patients develop allograft anti-GBM nephritis, when the antigenic defect of basement membranes is so common among them, is one of the unsolved mysteries of the disease.

Immunohistochemical studies of Samoyed hereditary nephropathy have recently been described by Thorner and his colleagues [42]. Male dogs with this X-linked dominant nephropathy develop GBM changes that are similar to those seen in men with Alport's syndrome [43]. A human anti-GBM autoantibody that bound linearly to normal dog GBM was found to lack any reactivity with the GBM of affected male Samoyeds, regardless of the age at which the dogs were examined. The GBM of the affected male Samoyed is indistinguishable from that of normal dogs at birth, and at three weeks of age the GBM is trilaminar with focal areas of lamina densa duplication. These observations suggest that the basket-weave alteration may arise through defective maintenance of the normal integrity of the lamina densa, rather than

being the result of failure of fusion of the glomerular endothelial and visceral epithelial basement membranes, as previously suggested [20].

THE NATURE OF THE GOODPASTURE ANTIGEN

Since the Goodpasture epitope, or epitopes, appears to be altered in some fashion in Alport's syndrome, identification of the molecule of which the epitope is a part may shed light on the basement membrane defect in Alport's syndrome. The Goodpasture epitope(s) has been localized to the carboxy-terminal, globular noncollagenous domain of basement membrane (type IV) collagen [44, 45], otherwise known as the NC1 domain. Type IV collagen is the structural backbone of all basement membranes, including GBM, and consists of at least two chains, the alpha 1(IV) and the alpha 2(IV) chains [46, 47]. These chains, which have similar amino acid sequences, are encoded by two very closely linked genes on the distal end of the long arm of chromosome 13 [48]. In many basement membranes, each type IV collagen molecule is a heterotrimer formed by two alpha 1(IV) and one alpha 2(IV) chains. When these chains are digested with bacterial collagenase, they yield the noncollagenous regions termed NC1 domains. Type IV collagen differs from other collagens in that it retains its noncollagenous carboxy-terminal domain rather than having it cleaved prior to secretion (for review see [49]). Separation of this material by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) reveals a monomer of 26 kilodalton (kDa) molecular weight that is derived from the alpha 1(IV) chain and a 24-kDa monomer derived from the alpha 2(IV) chain [50]. Such gels also contain protein bands of MW 48–54 kDa, which appear to be homo- and heterodimers of the NC1 monomers.

There appear to be additional basement membrane collagen chains likely related to type IV collagen and possibly representing alpha 3(IV) and alpha 4(IV) chains. Butkowski et al. [50], working with bovine GBM, have identified a third basement membrane collagen chain in which the Goodpasture reactivity of bovine GBM resides. This chain appears to be very similar to the chain represented by the 28-kDa NC1 monomer derived from human GBM, which has been shown by Kleppel and colleagues in our laboratory to carry the bulk of Goodpasture-reactive epitopes in human GBM [51]. It remains to be determined whether these additional chains are encoded by distinct basement membrane collagen genes.

STUDIES OF NONCOLLAGENOUS DOMAINS OF BASEMENT MEMBRANE COLLAGEN FROM ALPORT'S GBM

Since immunohistologic studies of Alport's GBM pointed to an alteration in the Goodpasture antigen, and by extension to the noncollagenous domains of type IV collagen, we studied Alport's GBM by methods previously used to study the Goodpasture antigen of normal GBM [52]. End-stage kidneys from three men with Alport's syndrome were available for study. Each individual was a member of a family in which the disease was transmitted in a fashion

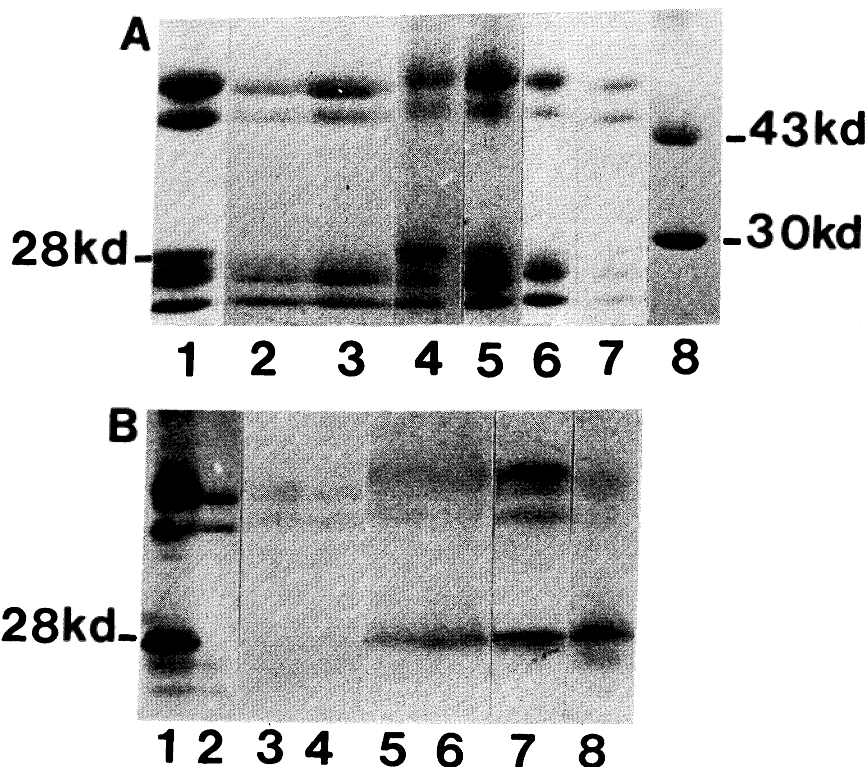

Figure 4-1. A. Collagenase-digested GBM separated by SDS-PAGE and stained with Coomassie Blue dye. Typical type IV collagen NC1 dimeric (43–54 kDa) and monomeric (24–28 kDa) subunits are observed in normal GBM (lane 1). The 28-kDa monomer bands are not present in Alport's GBM (lanes 2 and 3). GBM digests from patients with reflux nephropathy (lane 4), hypertensive nephropathy (lane 5), diabetic nephropathy (lane 6), and hemolytic–uremic syndrome (lane 7) appear normal, although the 28-kDa monomers are weakly stained in lanes 6 and 7. Lane 8 shows low-molecular-weight standards.
B. Immunoblots of the SDS-PAGE gels were stained wuth Goodpasture antibodies. Collagenase digests of normal GBM show typical staining of NC1 subunits (lane 1); the greatest reactivity is associated with the 28-kDa band. In Alport's GBM (lanes 2, 3 and 4), there is weak reactivity with NC1 dimers and the 24′ and 26-kDa monomers; no 28-kDa bands are seen. Collagenase-digested GBM specimens from patients with reflux nephropathy (lane 5), hypertensive nephropathy (lane 6), diabetic nephropathy (lane 7), and hemolytic–uremic syndrome appear similar to normal GBM. (Reproduced from The Journal of Clinical Investigation 80:263–266, 1987, by copyright permission of the American Society for Clinical Investigation.

consistent with X-linked dominant inheritance. Each kidney used showed no GBM reactivity with Goodpasture antibodies.

The cortices of these kidneys were minced and homogenized, followed by differential sieving, ultrasonic disruption, and centrifugation to isolate GBM. Complete separation of glomeruli and tubules was prevented, however, by the

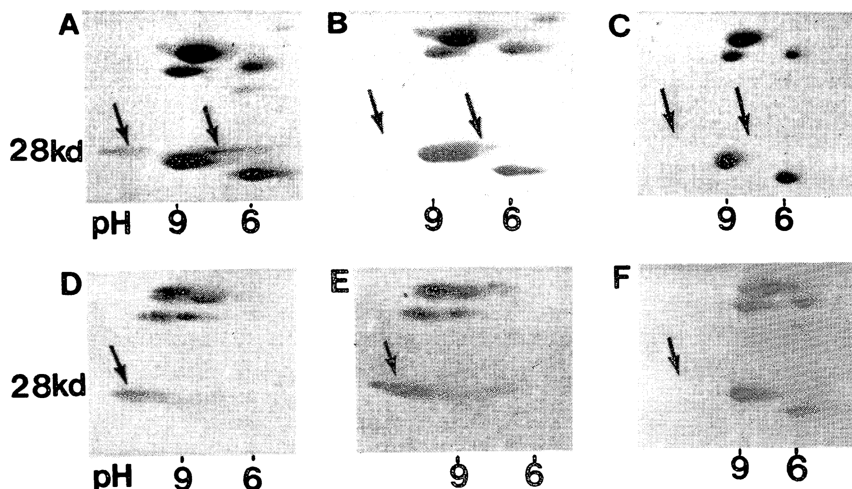

Figure 4-2. Collagenase-digested Alport's GBM and control GBM were separated in two dimensions, first by NEPHGE and then by SDS-PAGE. Gels were stained with Coomassie Blue dye (A–C) or immunoblotted with Goodpasture antibody (D–F). The normal distribution of NC1 subunits is seen in GBM from a patient with diabetic nephropathy (**A**) and in normal GBM (**B**). Note that two 28-kDa monomers are present (arrows), one that is very cationic (pH > 9.0) and one that is neutral in charge. Neither 28-kDa monomer is present in NC1 from Alport's GBM (**C**), and the dimer population appears simplified. Immunoblotting with Goodpasture antibody confirmed the absence of both 28-kDa monomers in the Alport's GBM digest (**F**). The cationic dimers are absent as well. Immunoblots of GBM digests from patients with hemolyticuremic syndrome (**D**) and congenital nephrotic syndrome (**E**) show a normal distribution of NC1 subunits. Note that in these preparations the most reactive monomer is the cationic 28-kDa component. (Reproduced from The Journal of Clinical Investigation 80:263–266, 1987, by copyright permission of the American Society for Clinical Investigation.)

advanced interstitial fibrosis in these kidneys. After collagenase digestion, the noncollagenous material was size-separated by SDS-PAGE. Some of the noncollagenous material was further separated on the basis of charge, using nonequilibrium pH gradient electrophoresis (NEPHGE).

The expected collection of monomers and dimers of the noncollagenous domains of basement membrane collagen was found in normal kidneys and in end-stage kidneys from patients with a variety of renal disorders (figure 4-1A). However, the Alport's kidneys all lacked the 28-kDa NC1 monomer (figure 4-1A). In addition, the dimer population appeared to be simplified. Immunoblots of the SDS-PAGE gels using Goodpasture antibodies confirmed the absence of the 28-kDa NC1 monomers in these preparations (figure 4-1B).

When collagenase-digested human GBM is separated in two dimensions, that is, by molecular weight and charge, two monomers of 28-kDa molecular weight are seen (figure 4-2A). One of the monomers has a neutral pI, while the other is very cationic. Further, there appear to be dimeric components aligned

with each of these 28-kDa monomers. Whether the parent molecules of these monomers represent additional type IV collagen chains (i.e., alpha 3 and alpha 4) or totally distinct polypeptides has not been resolved. The Alport's kidneys we studied lacked both 28-kDa monomers, and also lacked the dimeric species apparently composed of these monomers (figures 4-2C, 4-2F).

While these findings provide an explanation for the failure of Alport's GBM to bind Goodpasture antibodies, their significance is uncertain. It is possible that in Alport's patients the gene encoding the polypeptide of which the 28-kDa monomer is a part is deleted or significantly altered. If it is found that the two 28-kDa monomers represent two distinct collagen chains, a more complex explanation will be required. An alteration in a single gene that encodes two collagen chains via alternative splicing of mRNA, or via post-translational modifications, would be consistent with our findings. Alternatively, the collagen chain or chains may be normally synthesized but not integrated into GBM because of the absence of an enzyme which mediates binding of the collagen chain to other basement membrane components, for example the alpha 1(IV) or alpha 2(IV) chains of type IV collagen. The substrate for this enzyme could be the parent molecules of the 28-kDa monomers, or the molecules to which these chains bind in GBM.

Savage and her colleagues have also studied collagenase-digested Alport's GBM using methods similar to ours [53]. In their preliminary report, they indicated that each of the normal NC1 monomers and dimers was present in the GBM from the three Alport's patients studied. However, the reactivity of these moieties with human anti-GBM autoantibodies was altered, as shown by immunoblotting. Their observations would be consistent with an alteration in a basement membrane collagen gene resulting in the loss of normal epitopes, or with an enzymatic defect affecting the expression of normal epitopes. The alterations in antigenicity of NC1 components could also represent secondary phenomena, which however would be unique to Alport's syndrome. The reasons for the differences in our findings and those of Savage et al. are uncertain at present.

TRANSMISSION OF THE BASEMENT MEMBRANE DEFECT IN FAMILIES WITH ALPORT'S SYNDROME

In 1984, a young man with Alport's syndrome received a kidney transplant at the University of Minnesota. The donor was his HLA-identical but unaffected brother. Within several months following the transplant, the patient developed a severe crescentic glomerulonephritis associated with circulating as well as fixed antibodies to the GBM of his allograft [36]. Like the patient reported by McCoy and Wilson [37], our patient's anti-GBM antibody did not react in vitro with the GBM of his native kidney or with GBM of other Alport males. This antibody also reacted with the dermal–epidermal junction (DEJ) of normal skin but not with the patient's own DEJ or with the DEJ of other, unrelated males with Alport's syndrome [36].

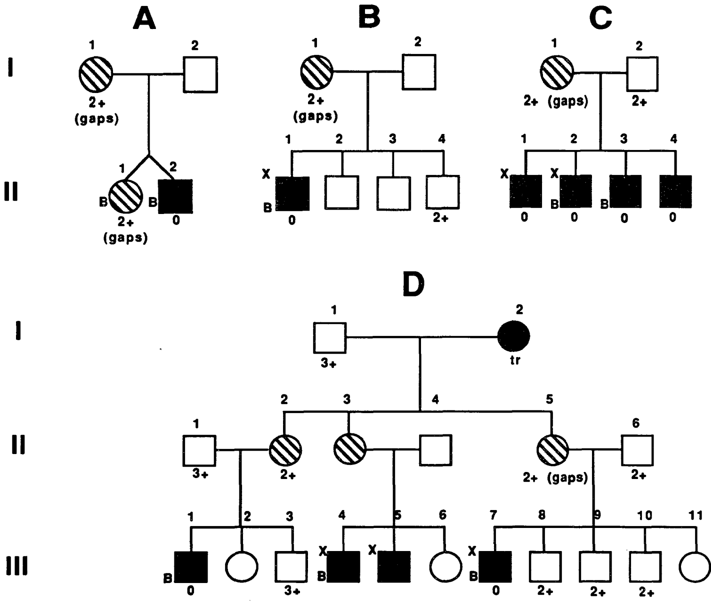

Figure 4-3. Pedigrees of four kindreds with Alport's syndrome. In these kindreds, serum from patient B-II-1, who developed anti-GBM nephritis in his allograft following renal transplantation, failed to stain the dermal–epidermal junction (DEJ) in skin specimens from males with Alport's syndrome. In contrast, IgG in the anti-GBM serum reacted intensely with DEJ from normal controls and unaffected kindred members by indirect immunofluorescence. Circles and squares denote males and females, respectively. Solid symbols indicate individuals with renal disease and sensorineural deafness; cross-hatched symbols represent subjects with renal involvement only. An X indicates a recipient of a renal transplant, and B a subject with renal ultrastructural alterations typical of Alport's syndrome. Intensity of DEJ fluorescence after staining with the anti-GBM serum is described below the symbols representing family members. Fluorescence intensity was graded as 0, trace (tr), 1+, 2+, and 3+. *Gaps* refers to regions of nonreactive DEJ spearating regions of reactive DEJ. (Reproduced from The Journal of Clinical Investigation 78:1035–1044, 1986, by copyright permission of the American Society for Clinical Investigation.)

We took advantage of the DEJ reactivity of this patient's serum to study the transmission of the basement membrane antigenic defect in families with Alport's syndrome. Members of five families were studied initially; we have now examined skin from individuals in 20 kindreds with the disorder. Our findings can be summarized as follows (figures 4-3 and 4-4): 1) unaffected males and females show normal binding of the Alport's anti-GBM serum to their DEJ; 2) DEJ from affected males in the majority of the families (16 of 20) lacks reactivity with the serum; and 3) affected females exhibit either normal binding of the serum, or a mosaic pattern of binding in which segments of DEJ that react normally with the serum are present adjacent to segments that show no reactivity. One woman with end-stage renal disease showed minimal binding of the serum to her DEJ.

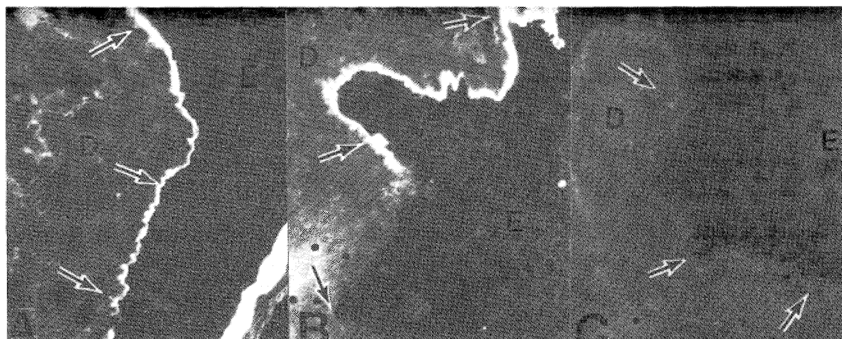

Figure 4-4. Skin specimens from three members of an Alport's kindred were stained with the Alport's anti-GBM serum described in figure 4-3 and the text. (**A**) Note brilliant fluorescene (arrows) of DEJ in an unaffected male (X 475). (**B**) Reactivity of DEJ is discontinuous in an affected female. Nonreactive DEJ is designated by the bracket (X 390). (**C**) There is no DEJ fluorescence in this affected male, who is the child of the individuals whose skin is shown in (A) and (B) (X 370). Arrows, DEJ; E, epidermis; D, dermis. (Reproduced from The Journal of Clinical Investigation 78:1035–1044, 1986, by copyright permission of the American Society for Clinical Investigation.)

We interpreted these findings as being consistent with X-linked dominant transmission of a defect in the epitope recognized by an antibody in our patient's anti-basement membrane serum. The DEJ synthesized by the keratinocytes of an affected male completely lacks the target antigen because of an alteration in an X-linked gene. The mosaic pattern seen in many of the affected women could arise if clones of keratinocytes having an active normal X-linked gene were adjacent to clones in which the abnormal gene was active. This hypothesis is consistent with the random inactivation theory of Lyon [54–56], which has been confirmed in several experimental and clinical settings [57–60], as well as with data indicating that extensive intermixing of epidermal cell clones followed by coherent clonal growth occurs during development of the skin [61–63].

Several groups of investigators have recognized that Alport's families can be distinguished on the basis of the age when affected males develop end-stage renal disease [64–66]. Absence of DEJ reactivity with our antibody probe has been found in families in which affected males progress rapidly to renal failure (types I and II Alport's syndrome, according to the classification of Atkin and colleagues [67]), as well as in type III families, where the development of renal failure in affected males is delayed until the fourth or fifth decade of life (Kashtan et al., submitted for publication). This finding suggests that the basement membrane defects in juvenile and adult-onset renal failure families are similar, perhaps arising from slightly different mutant alleles at a single locus. An alternative explanation is that there is a single defective allele, and that the difference in the rate of progression reflects the genetic back-

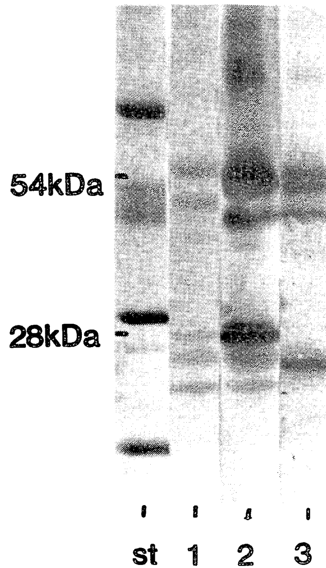

Figure 4-5. Coomassie Blue stain and immunoblots of collagenase-digested normal human GBM separated by SDS-PAGE. Lane st: Coomassie Blue stain of low-molecular-weight standards. Lane 1: Coomassie Blue stain of normal GBM, showing dimers (43–54 kDa) and monomers (24–28 kDa) of type IV collagen NC1 domain. Lane 2: Immunoblot of normal GBM using Goodpasture antibody. Note reactivity with NC1 dimers and monomers, in particular the 28-kDa monomer. Lane 3: Immunoblot of normal GBM using the Alport's anti-GBM serum. Reactivity in the monomer region is confined to the 26-kDa monomer, while reactivity with each of the dimer bands is observed. (Reproduced from The Journal of Clinical Investigation 78: 1035–1044, 1986, by copyright permission of the American Society for Clinical Investigation.)

ground of the affected individual, perhaps involving a predisposition to the development of hypertension, as in diabetic nephropathy [68, 69], or inherited differences in the susceptibility of damaged glomeruli to the development of glomerulosclerosis [70].

In 4 of the 20 families, affected males retain DEJ reactivity with the antibody probe. These include a family with X-linked dominant disease [36], two families in which the defect appears to have occurred as a new mutation in a single male, and one family in which the pattern of transmission suggests X-linked dominance but is also consistent with autosomal dominance. We have not studied, because we have not encountered, any families with unequivocal autosomal dominant transmission of Alport's syndrome (type VI by the Atkin classification).

The reactivity of our patient's antibody with collagenase digests of normal human GBM has been studied by immunoblotting (figure 4-5) [36]. The antibody recognizes a 26-kDa protein, which may represent the NC1 domain of the alpha 1(IV) chain of type IV collagen. It is possible that the 26-kDa protein

identified by the antibody is a collagenase-resistant portion of another, as yet incompletely separated and characterized, basement membrane collagen [71, 72]. We have also examined the reactivity of the anti-GBM antibody studied by McCoy and Wilson [37]; vide supra). This antibody differs from our patient's antibody in that it does not bind to normal DEJ, Bowman's capsule, or tubular basement membranes. Further, immunoblotting of collagenase-digested normal GBM shows that the antibody recognizes principally the 28-kDa NC1 monomer. Thus it appears that the immune responses of transplanted Alport's patients to normal GBM may be heterogeneous.

These observations introduce new factors into the already complex Alport's equation. We have found abnormalities involving two, and perhaps three, distinct peptides in Alport's GBM: 1) a 28-kDa peptide that appears to represent the noncollagenous domain of a partially characterized basement membrane collagen chain and that separates into cationic and neutral components by two-dimensional electrophoresis; and 2) a 26-kDa peptide that may be the noncollagenous domain of the alpha 1(IV) chain or of a distinct, also uncharacterized, basement membrane collagen. The possibility that there is a structural defect in the gene encoding the alpha 1(IV) chain can probably be dismissed, since the gene for this chain is located on chromosome 13 [48] while the majority of Alport's families have an X-linked dominant disorder [65, 66]. Could the gene for the collagen chain carrying the 28-kDa noncollagenous domain be deleted in Alport's males? If so, the absence of this chain from basement membranes would have to result in the loss of an epitope on another collagen chain to account for the failure of our patient's antibody to react with Alport's basement membranes (vide supra). Against this hypothesis is the fact that monoclonal antibodies that recognize the 28-kDa noncollagenous domain do not react with normal DEJ, suggesting that the presence of this peptide in basement membranes is not required for expression of the epitope recognized by our patient's antibody (Kleppel et al., submitted for publication).

Another hypothesis would suggest that expression of the 28-kDa parent molecule is confined to certain basement membranes, including the glomerular basement membrane and the basilar membrane of the cochlea and certain basement membranes of the eye (Kleppel et al., submitted for publication). Integration of the 28-kDa parent protein into these basement membranes may require the activity of an enzyme that catalyzes binding to other basement membrane components, such as the alpha 1(IV) chain. This enzyme might also create an epitope on the NC1 domain of the alpha 1(IV) chain in the process of carrying out the binding reaction. Absence of this enzyme could then result in 1) failure of the 28-kDa parent molecule to become integrated into basement membranes, and 2) the loss of a normal epitope on the alpha 1(IV) chain. A problem with this hypothesis is that it suggests that the enzyme is also active in the DEJ, although the 28-kDa molecule does not appear to be part of the DEJ. Finally, it may be that the alterations observed in basement membrane collagens are secondary consequences of a defect in a gene encoding an unrelated protein.

Clearly we are still some distance away from a fundamental understanding of the basement membrane anomalies of Alport's syndrome. Ultimately this task may require isolation of the defective gene and expression of its gene product. As a prelude to isolation of the gene, efforts to localize the gene by analysis of linkage to restriction fragment length polymorphisms are well under way [73].

What might be the consequences of alterations in the NC1 domain(s) of basement membrane collagen? Studies of extracellular matrices have shown that type IV collagen molecules interact with each other to form a complex polygonal network that includes end-to-end and lateral associations involving the NC1 domains [74–76]. In vitro, these associations can be prevented by pepsin treatment, which cleaves the NC1 domain from the collagenous portion of the type IV collagen chain, or by antibodies directed against the NC1 domain [77, 78]. An alteration in the integrity of these intermolecular associations could result in structural and/or functional deficits in basement membranes. The NC1 domain can also bind to basement membrane components such as heparan sulfate proteoglycan and nidogen in vitro [79, 80], so that NC1 defects could alter interactions between basement membrane collagens and other matrix molecules.

One of the most intriguing of the Alport's mysteries concerns the relationship between nephropathy and sensorineural hearing loss. Why do people (especially males) with Alport's syndrome suffer deafness? How do they differ from individuals with clinically and histologically identical renal disease who have normal hearing? The once-popular hypothesis that hearing loss resulted from ototoxins elaborated or not excreted by the Alport's kidney is no longer tenable. No Alport's-specific ototoxin has been identified, and reports of improved hearing after renal transplantation [81] have been contradicted by studies showing either no enhancement of hearing posttransplant or similar improvements in hearing in patients with non-Alport's kidney disease [82, 83]. There are two other hypotheses that are very attractive, although at present there are few data to support or contradict either of them. First, perhaps basement membranes of the cochlea are biochemically altered in a fashion similar to GBM and other basement membranes, resulting in an abnormal response to sound, or perhaps in scarring of critical cochlear structures. Our finding that the DEJ shares an antigenic abnormality with GBM in Alport's patients indicates that the biochemical defect is diffusely distributed, although the consequences of the defect must vary markedly from organ to organ. Basement membranes of normal human cochlea, such as the basilar membrane of the organ of Corti, express epitopes that appear to be important in Alport's syndrome, such as the antigen recognized by our Alport's patient's antibasement-membrane antibody and antigens identified by monoclonal antibodies to the 28-kDa noncollagenous domains of basement membrane collagen (Kleppel et al., submitted for publication). These observations will be difficult to interpret until more is known about the functions of cochlear basement mem-

branes. Even if Alport's cochlear basement membranes were to exhibit the same antigenic abnormalities that characterize epidermal and glomerular basement membranes, the actual cause of hearing loss may not be related to the antigenic defect. Postmortem histologic studies of the Alport's cochlea, which of necessity are performed late in the course of the disease, have produced variable findings, although atrophy of the stria vascularis and haircell degeneration have frequently been observed [84–88]. The specificity of these findings is uncertain, and they may be related to uremia, ototoxic drugs, or preterminal events.

A second hypothesis suggests that in families with the Alport's nephropathy and deafness, a deletion on the X-chromosome affects the activity of two tightly linked genes, one involved in basement membrane synthesis and organization, and another that is critical to maintenance of normal cochlear structure and function. This hypothesis is supported by the existence of families in which sensorineural hearing loss is transmitted as an X-linked trait, in the absence of renal disease [89, 90]. There appears to have been no systematic cytogenetic examination of Alport's chromosomes using high-resolution cytogenetic techniques. The postulated deletion may be too small, in any case, to be visible, and may only be discovered when markers very closely linked to the Alport's gene have been obtained.

Studies currently underway or contemplated may provide data in favor of one or the other of these hypotheses. For example, if families with renal disease but normal hearing (type IV Alport's syndrome) display the antigenic defect of basement membranes seen in most families with classical Alport's syndrome, then one may surmise that the nephropathy and deafness involve two distinct genes. The finding of normal DEJ antigens in such families would support the argument that types I–III Alport's syndrome (juvenile or adult-onset renal failure with deafness) arise from mutant alleles at one locus, and type IV (adult-onset renal failure without deafness) involves a different locus. A second approach that might generate useful information would be a linkage study of X-linked sensorineural hearing loss. Localization of this gene to the region where the Alport's gene resides would make plausible the idea that a single deletion or rearrangement could involve both genes.

AMYLOID P IS NOT PRESENT IN ALPORT'S GBM

Amyloid P component is a glycoprotein found in association with amyloid deposits, and is apparently identical to serum amyloid P component, a normal plasma constituent [91]. In the normal human kidney, amyloid P is found in GBM, mesangium, and arterial walls [92]. Recently Melvin et al. [93] observed that antibodies to amyloid P component failed to bind to GBM of Alport's males whose GBM also lacked reactivity with Goodpasture antibodies. However, amyloid P was present in the glomerular mesangium and in the arterial walls of these patients.

These findings raise intriguing questions. First, to what GBM component(s)

does amyloid P bind? The amyloid P in vascular walls is known to bind to elastic microfibrils [94] whereas the binding site(s) for amyloid P in the mesangium and GBM is unknown. Of interest, collagenase digestion of GBM releases amyloid P that it associated with other peptide fragments [92]. Perhaps the abnormal Alport's NC1 domain fails to interact normally with circulating or locally synthesized amyloid P, preventing its incorporation into GBM. Second, what are the consequences of the loss of amyloid P from the GBM? It is difficult to answer this question because of the paucity of information regarding the normal functions of amyloid P. However, there is evidence that amyloid P can inhibit the activity of pancreatic elastase in vitro [95]. One can speculate that the Alport's GBM may be more vulnerable to proteolytic attack because of the absence of amyloid P.

CONCLUSIONS

As noted at the beginning of this review, there has been significant progress in our understanding of the Alport's lesion, but there is still much to learn. We expect that biochemical and molecular genetic approaches will continue to expand our knowledge of Alport's syndrome, and that eventually its mysteries will be unraveled.

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5. THE INHERITANCE OF ALPORT'S SYNDROME

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INTRODUCTION

In 1902, Guthrie described an English family in which 12 of 15 members from two generations had "idiopathic" hematuria. He reported that in every affected individual the hematuria increased intermittently, usually in association with fever, malaise, headaches, backaches and pains in the legs. Precipitating factors included infections (often of the upper respiratory tract) and exposure to extremes of temperature. Urinalysis also revealed proteinuria in most cases [1].

The same family was examined a decade later [2], and reported again by Hurst (anglicized from Hertz) 11 years after that [3]. By then, some of the patients had died from uremia, and Hurst noted that three siblings (two male and one female) were deaf. The following year Eason drew attention to the severe course of the disease with early death in males, in contrast with its relatively benign course in females [4].

By the time Alport reported the same family three years later, the pedigree extended over three generations [5]. He found that almost all the family members with hematuria also had some degree of nerve deafness, and he was the first person to draw attention to this combination as a specific clinical syndrome. He also recognised that hematuria was the most common presenting symptom, and re-emphasized the contrast in severity of the disease between males and females.

Between 1929 and 1959, 15 families with similar symptoms were described,

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and many authors noticed that these families included females who were asymptomatic carriers of the trait, which led to a discussion of the hereditary aspects of this type of familial nephritis [6]. In 1961 Williamson reported two more families and suggested the eponym *Alport's syndrome* [7].

In 1964, two families from Athens were reported [8]. The author commented that the disease did not seem as rare as was originally thought, and emphasized that the bad prognosis (especially in males) and the hereditary nature of the disease justified thorough clinical examination of the relatives of every patient diagnosed.

During the 1960s and early 1970s, 150 additional families of all races were described in different parts of the world. In 1968 the original family that Alport had studied was retraced and found to have "rid itself of Alport's disease", since no new cases had appeared [9].

Alport described the specific disease that affected this family very clearly, but only a few authors have applied strict diagnostic criteria before using the eponym [10]. Many workers have used the label *Alport's syndrome* for families with other inherited nephritides without deafness [11, 12, 13]. Clinical and genetic analysis of this heterogeneous group proved complicated and, not surprisingly, produced evidence for genetic heterogeneity.

THEORIES OF INHERITANCE IN ALPORT'S SYNDROME: HISTORICAL REVIEW

Family P—X-linked eventually

A large Mormon family in Utah (often referred to as Family P) has been the subject of repeated genetic analyses. Perkoff defined individuals as affected if they had one or more of the following: hematuria, proteinuria, pyuria and casts. He found two affected and 15 unaffected sons of affected fathers, and 18 affected and four unaffected daughters of affected fathers. He postulated partial X-linkage with crossing-over between the X-chromosome and the Y-chromosome [14, 15]. Seven years later the number of normal sons and affected daughters born to affected fathers had increased [16], and an hypothesis of dominant complete sex-linkage was proposed, with some other disease accounting for the two cases of male-to-male transmission [17]. Graham also disagreed with the idea of partial sex-linkage, but proposed that Family P showed autosomal dominant inheritance with incomplete penetrance and sex influence, being more severe in males, with intrauterine death of half of the affected males [6]. The discussion continued in the correspondence columns of the American Journal of Human Genetics [18, 19].

In 1961, Shaw reanalyzed data from Family P and another kindred, and favored Graham's suggestion [20]. In addition, he postulated an abnormality at gametogenesis of non-random disjunction leading to preferential segregation of the chromosome bearing the mutant gene—i.e., in oogenesis the chromosome carrying the disease gene went to the oocyte (rather than to the polar body), and in spermatogenesis the mutant chromosome segregated preferentially

with the X-chromosome. There was considerable support for Shaw's hypothesis [21–24], with a modification suggesting that when the mother was symptomatic, a higher proportion of her offspring was affected [25].

Mayo was the first author to emphasize that groups of families being analyzed together must demonstrate clinical homogeneity for genetic analysis to be valid [26]. He found that the pedigrees used in support of Shaw's hypothesis were heterogeneous, invalidating the genetic conclusions drawn.

When Family P was reinvestigated using hematuria rather than pyuria or proteinuria as the essential diagnostic criterion, the cases of male-to-male transmission were eliminated, and a simple 2:1 ratio of affected females:males, compatible with simple X-linked inheritance, was revealed [27].

Subsequently Hasstedt [13] used segregation analysis to confirm X-linked inheritance in Family P, with a gene penetrance of 1.0 in males and 0.85 in females. After correcting for ascertainment bias, she was unable to confirm many of the abnormal segregation ratios reported previously.

X-linked inheritance in other families

In addition to Family P, several other families have been reported as demonstrating X-linked inheritance [28–30]. One of the earliest, in which deafness was associated with the renal problems, had no male-to-male transmission, and all the daughters of affected fathers inherited the gene [31]. However, in another family, reported the following year, there were six apparently unaffected daughters born to affected fathers [32]; but the significance of reduced gene penetrance was emphasized, since this family also included four apparently normal females who subsequently transmitted the disease to their offspring.

We have suggested the use of four diagnostic criteria that enable identification of patients affected by the same disease characteristics as Alport's original family. We diagnose Alport's syndrome in any family that demonstrates at least three out of four of the following characteristics.

1. Positive family history of haematuria with or without renal failure
2. Characteristic electron microscopic changes of the glomerular basement membrane on renal biopsy
3. Diagnostic ophthalmic signs
4. High-tone sensorineural deafness

These features are discussed in more detail later. In our study, 41 families with "classic" Alport's syndrome were examined and all the pedigrees were found to be compatible with X-linked inheritance [10].

Autosomal dominant inheritance

Thirty-one published pedigrees show male-to-male transmission of renal disease called "Alport's syndrome", but the diagnostic criteria used vary con-

siderably [10, 33]. In particular, only a minority of those with evidence of renal disease had any hearing impairment, and this was usually either atypical or not specified. None of the affected individuals had any of the diagnostic eye signs [34], but a variety of non-specific eye abnormalities (e.g. cataract) was described [20, 35, 36]. In some of the pedigrees affected individuals had renal biopsies, but the histological evidence for Alport's syndrome was poor, as most of the abnormalities described were patchy and non-specific changes of the glomerular basement membranes.

Generally, families that have an autosomal dominantly inherited nephritis (with male-to-male transmission) appear to be affected by a renal disease that often presents with hematuria, and may proceed to chronic renal failure in either sex; but neither the characteristic high-tone sensorineural deafness nor the diagnostic eye signs are present. We have proposed that the eponym is inappropriate in these cases, and that they should be labeled *hereditary nephritis without deafness* [33].

Autosomal recessive inheritance

The evidence for an autosomal recessive form of Alport's syndrome is scanty in the 12 families reported as representing this category. In two of the families, the mothers had abnormal urinalysis that was not fully investigated [37, 38], and in a third family the father was deaf [39, 40]. In the remaining families either the nephritis is atypical (and not accompanied by deafness) or the pedigree data are incomplete and the mothers have not been fully excluded as carriers [28, 30, 37, 39, 41].

Hasstedt categories

In a review of 23 families from Utah with inherited nephritis, Hasstedt proposed six categories for the classification of pedigrees including two autosomal groups, despite the fact that there was no male-to-male transmission in any of her pedigrees [13]. The diagnosis of Alport's syndrome was made on the basis of urinalysis and renal biopsy alone.

Sporadic cases

The difficulty in diagnosing an inherited disease in the absence of a positive family history means that sporadic cases are less likely to achieve the correct diagnosis [42]. Nevertheless, Shaw calculated a new mutation rate among affected individuals of 18%. A comparatively high rate of new mutations is to be expected with a disease which tends to reduce the fertility of affected males because of the comparatively early age at death in untreated males [43].

Conclusion

A review of the literature on the genetics of Alport's syndrome is complicated because the diagnostic criteria used have varied, and some authors have pooled

data from families with clinically distinct renal diseases. It is hardly surprising that there appeared to be considerable genetic heterogeneity.

From the literature, the evidence that "classic" Alport's syndrome (i.e., hereditary nephritis with deafness) is X-linked is very strong. The families reported with autosomal dominant nephritis have less typical disease; and, in particular, do not have the characteristic deafness and ophthalmological manifestations. In the families with "classic" Alport's syndrome which were originally thought to demonstrate autosomal inheritance, cases of male-to-male transmission can be excluded by using stricter diagnostic criteria.

There are several apparently unaffected daughters born to affected fathers; but as most of these were children at the time of examination, and the gene penetrance of X-linked conditions is often less than 100% in females, this does not exclude X-linked inheritance.

Alport made no attempt to define the pattern of inheritance in his original family, as none of the affected males had reproduced. Many subsequent statistical analyses have also been handicapped by the paucity of offspring born to affected males, who tended to die young prior to the availability of renal replacement therapy.

In conclusion, the evidence from the literature for genetic heterogeneity in Alport's syndrome is based on the analysis of a group of families with considerable phenotypic variation. In view of the fact that Alport emphasised that deafness is an integral part of the syndrome, perhaps the eponym should be reserved for this specific subgroup of hereditary nephritides.

CLINICAL FEATURES

Positive family history

Several authors have emphasised the importance of obtaining an accurate and detailed family history in any patient found to have glomerulonephritis [6, 13, 30, 44, 45, 46]. Strenuous attempts should also be made to counsel and examine the close relatives. In a recent German study, only 20% of patients with various types of familial glomerulonephritis were aware of renal disease in their relatives, and the majority of affected relatives were diagnosed *de novo* after systematic examination and investigation. Ten percent of cases of glomerulonephritis were found to be familial, and of these, half were diagnosed as classic Alport's syndrome [44]. The absence of other affected individuals in a family does not exclude genetic disease however, as the patient may represent a spontaneous gene mutation.

Pathological evidence of Alport's syndrome

The renal pathological lesions in Alport's syndrome were considered to be non-specific for many years [23, 47–51]. In the early 1970s, three groups of pathologists drew attention to various definite ultrastructural lesions of the glomerular basement membrane (GBM) [40, 52 ,53]. Typically, the GBM splits

and the lamina densa becomes thicker, reaching a diameter of up to 510 nm (normal 200–350 nm) [54]. Instead of a homogeneous band, there is a network of small, anastomosing strands about 100 nm thick. Lucent areas within the lamina densa contain small electron-dense particles of various sizes (average 50 nm). The ultrastructural pattern can be very complex and the GBM lesions can be patchy, alternating with segments of normal thickness [49], especially in females and children. Serial renal biopsies reveal that the lesions may be absent on the first biopsy and appear subsequently [55, 56]. Many authors have discussed whether the GBM changes are diagnostic, or just characteristic of Alport's syndrome. Diffuse GBM thickening and splitting is highly suggestive of Alport's syndrome when immunofluorescent studies are negative [30, 39, 40, 46, 57, 58], and the simultaneous inclusion of electron-lucent areas containing granulations appears to be characteristic of Alport's syndrome.

If the biopsy of a newly presenting patient suspected of having Alport's syndrome is equivocal, then it is often useful to obtain the results of biopsies performed on close relatives, particularly males, in order to clarify the diagnosis. It may even be possible to avoid performing renal biopsies on children with hematuria, if sufficient, well-documented evidence of Alport's syndrome is available in other members of the family. The immunogenetics of the glomerular basement membrane are discussed in chapter 4 of this volume.

Ophthalmic signs

Ocular disorders, mainly involving the lens, were described originally by Sohar [59, 60]. Subsequently, the association of hereditary nephritis and deafness with eye abnormalities was reported by several authors [35, 47, 61–68]. A few families with inherited nephritis and eye anomalies without deafness have been reported [69], but this is unusual [39].

Bilateral anterior lenticonus is the most specific abnormality in Alport's syndrome [34]. Nielsen found that all reported cases of lenticonus during a 13-year period had evidence of nephritis, and concluded that anterior lenticonus was diagnostic of Alport's syndrome [70]. Anterior lenticonus is associated with gradual deterioration of vision and the development of axial myopia. Posterior lenticonus is less common in Alport's syndrome [34]; and spherophakia, which has also been found [24, 60], may just represent marked lenticonus.

Many lens opacities have been described in association with Alport's syndrome [66, 71], but no lens opacity appears to be specific for Alport's syndrome [34]. Up to 75% of patients under 40 years with a variety of renal diseases develop posterior subcapsular lens opacities after renal transplantation [34], and there are only isolated reports of lens opacities developing in patients generally on hemodialysis who were not receiving steroids [72].

Macular flecks were reported initially by Castleman [47], and characterized as a few flecks in the perifoveal region [73] and also in a much more profuse distribution around the fovea [74]. Midperipheral flecks are less frequent [75],

and fluorescein angiography of the macular region is always normal [76]. The ophthalmic signs may precede deterioration in renal function [76].

Several authors have commented on a weakened macular reflex [24, 76], and this may be the only abnormality in children [57].

Govan [34], in his definitive review, concludes that the diagnosis of Alport's syndrome can be made on the presence of three characteristic features:

1. Anterior lenticonus
2. Macular flecks
3. Peripheral coalescing flecks

The absence of these features, however, does not exclude the diagnosis.

High-tone, sensorineural deafness

The development of sensorineural deafness in a patient with hematuria is highly suggestive of Alport's syndrome, even in the absence of a positive family history. The hearing loss is bilateral, but may only be detected by audiometry. Deafness may be apparent during the first decade [57], particularly in boys. Serial audiograms in childhood often show progressive deterioration [64, 77, 78], necessitating a hearing aid [57, 62]. In adults, the hearing impairment is usually static [49], and most patients retain some hearing capacity [79]. Hearing improvement has been described after a successful renal transplant [80], but this may represent a non-specific improvement in deafness attributable to uremia [81].

Electron microscopic studies have shown a multilayered basement membrane of the vas spirale [82] consistent with the GBM abnormalities.

Other extrarenal abnormalities associated with Alport's syndrome

Several extrarenal abnormalities, in addition to deafness and ocular signs, have been described in Alport's syndrome. Hyperprolinemia and hyperaminoaciduria have been reported in a few families with familial nephritis, including some with Alport's syndrome as defined above, [57, 62, 83], but the association may be coincidental [57, 79].

The association of hereditary macrothrombocytopenia, nephritis with hematuria and proteinuria, and deafness was reported first by Epstein [84], and several authors believe that it is significant. We have seen a patient with Alport's syndrome and thrombocytopenia, however, in whom the Alport's syndrome was inherited from his mother and the thrombocytopenia from his father, and so, in this case at least, the simultaneous occurrence of the two features is purely coincidental.

The significance of antithyroid antibody abnormalities in Alport's syndrome is also uncertain, since the two conditions may segregate independently within the same family [85].

CLINICAL FINDINGS

We have studied 188 affected individuals from 41 families which fulfill at least three of the four criteria listed above. It has been recognized for many years that males and females with Alport's syndrome have a different clinical course [5, 79]. The disease progression is more predictable in males, with hematuria in early childhood (increasing during intercurrent infections), development of progressive sensorineural deafness during school years, and chronic renal failure and eye changes in the late teens or early twenties. Females have a more variable clinical course, and many remain asymptomatic into their eighties (with microscopic hematuria), but a few may be as severely affected as males.

Presentation

Macroscopic hematuria was the most frequent cause of presentation in both sexes (67% of males and 36% of females) at an average age of 3.5 years (range 0.1–18) and 9.1 years (range 1.75–40), respectively. Other common presentations include incidental finding of microscopic hematuria on routine analysis (10% of males, 8% of females); hematuria detected after screening because of family history of Alport's syndrome (4% of males, 32% of females); chronic renal failure (10% of males, 6% of females); the nephrotic syndrome (5% of males); and hypertension (2.5% of males, 5% of females).

Renal function

All affected individuals (both male and female) for whom we were able to perform urinalysis ourselves had microscopic hematuria. Of the 80 males whose urinalysis was either performed by us or well documented, 78 (97.5%) also had varying degrees of proteinuria. The two boys with isolated hematuria were aged 4 and 10 years. Sixty-three out of 94 females (67%) had proteinuria in addition to the hematuria (the average ages of females with and without proteinuria were not significantly different, and the females with isolated hematuria ranged in age from 6 to 82 years [86]). We have not seen any patient with Alport's syndrome develop proteinuria prior to the onset of hematuria, and we agree with the findings of Ferguson and Rance that affected females develop hematuria by the age of 20 years [87].

Seventy-five percent of males and 33% of females had hypertension, and the average age at which this was diagnosed was 17 years (range 10–35 years) and 32 years (range 17–55 years), respectively. Seventy-five percent of males and 21% of females had reduced renal function, and 65% and 15% respectively were in chronic renal failure. Of those with impaired renal function, the average age at which the creatinine began to rise was 18 years (range 10–35 years) and 31 years (range 10–48 years), respectively.

The figures for age at which the serum creatinine began to rise, and age at diagnosis of chronic renal failure, were known to the nearest year. This gave an estimate of the interval between the two of 2.2 years in males and 8.6 years in females. The exact serial serum creatinine results were available for 16

males, however, and when plotted against time showed an average time of 16 months for the creatinine to rise from 200 to 1000 μmol/L (2.3 to 11.4 mg/dl) [86]. Unfortunately there were not sufficient data to make the equivalent calculation for women.

The oldest male and female with normal renal function were 49 and 85 years old, respectively. The 49-year-old male, who also has lenticonus, has a 10-year-old daughter with biopsy-proven Alport's whose creatinine is 130 μmol/L (1.5 mg/dl). He is an exception, however, and the next oldest male who still has normal renal function is only 22 years old.

Thirty-two percent of males and 3% of females have been transplanted, and of these, 81% and 100% of grafts were functioning at the time of review.

Eye signs

Seventy-two percent of males and 38% of females examined had evidence of lenticonus, macular flecks, or both. In males, the eye signs usually appeared at about the time renal function began to fail, but in females the relationship between the two was more variable.

Hearing

Eighty-three percent of males had the typical high-tone sensorineural deafness, with an average deficit of −66 dB. Fifty-seven percent of females were deaf with an average loss of −50 dB.

It is inevitable that studies of this type will tend to overestimate the severity of disease in females, since those who are only minimally affected may not present, and will not necessarily be detected by family screening programs unless they have affected sons or brothers. Nevertheless, the findings of a more severe and uniform clinical progression of a genetic disease in affected males, contrasting with much more phenotypic variation in heterozygous females, is typical of X-linked disease. It has been postulated that random inactivation of the X-chromosome in each cell (Mary Lyon hypothesis) might explain the variability of gene expression in females [88].

ANALYSIS OF OFFSPRING

Eighty-six females have had 217 children, of whom 115 are affected (55 males and 60 females) and 102 are normal (44 males and 58 females). Eight males have reproduced, and they have had 11 affected children (all females) and four normal children (all males). There has been no male-to-male transmission of the disease, and all the daughters of affected men are affected [10]. This pattern strongly suggests X-linked inheritance.

The distribution in the sex ratio of offspring of affected males is unremarkable in such a small sample, but may be influenced by ascertainment bias. Five of the daughters were born to two affected males, both of whom died over 30 years ago. The clinical status of the daughters was only ascertained after they had had affected sons. Affected males of the same generation who only had normal sons would not have been detected by this study.

We calculated the reproductive fitness of males to be 0.516, and the incidence of affected males among all males to be 1.7×10^{-5}. Using these figures, we obtained a mutation rate (μ) per gene per generation of 2.74×10^{-6} [86], which is well within the range of μ estimated for other X-linked loci.

Of patients newly presenting with Alport's syndrome, we found that 6% had no affected relatives, and therefore were presumed to represent new gene mutations.

GENE LINKAGE STUDIES

We have screened 24 families with seven X-chromosome DNA probes, and confirmed linkage to probes S21 (DXS 17) and p19-2 (DXS 3), which are located on the long arm of the X-chromosome [86, 89]. Two other groups have published results of gene linkage studies. Brunner studied three European families that fulfilled our diagnostic criteria and found linkage to S21 [90]. The combined maximum LOD score for S21 from the English and Dutch studies is 8.34 at $\theta = 0.05$, with LOD minus 1 confidence intervals of 0.01–0.14 [89]. Three families studied in Utah [91, 92] showed linkage to p19-2 (DXS 3) and the combined maximum LOD score for p19-2 from the English and American studies is 9.50 at $\theta = 0.2$, with confidence intervals of 0.01–0.14 [89].

The finding of linked DNA markers on the X-chromosome confirms the pedigree data, demonstrating that classic Alport's syndrome is an X-linked disease. Finding a significant LOD score in a relatively large sample also suggests that, on these strict diagnostic criteria, Alport's syndrome is a homogeneous condition.

CONCLUSIONS

Alport was not the first author to describe the syndrome that bears his name, but he emphasized that the occurrence of sensorineural deafness with hematuria represents a specific clinical syndrome. Subsequent authors have diagnosed Alport's syndrome in families with inherited nephritides without the characteristic extrarenal manifestations, and it is not surprising that clinical and genetic analysis of this heterogeneous group produced evidence for genetic heterogeneity.

Careful review of the literature suggests that the use of strict diagnostic criteria, together with thorough investigation of apparently normal females, weakens the evidence for autosomal forms of Alport's syndrome considerably.

We believe that our criteria enable the identification of families affected with the same disease characteristics as Alport's original family. The sex difference in the clinical course of the disease is typical of X-linked inheritance, and analysis of the offspring of affected individuals is entirely compatible with X-linked inheritance.

The results of three gene linkage studies suggest that the gene is localized in the middle of the long arm of the X chromosome; and, once closer-linked

markers are identified, carrier testing and prenatal diagnosis will become available.

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6. GENETICS OF FAMILIAL HEMATURIA

PAIGE KAPLAN, MARY ELLEN TURNER, BERNARD S. KAPLAN

In a scholarly essay entitled *Nature, Nurture, and Human Affairs*, Scriver (1981) discussed the biologic origins of rickets in terms that can be modified for the better understanding of many other conditions, symptoms, or abnormalities in which a common phenotype (for example hematuria) may be the result of environmental factors, genetic factors, or an interaction between the two [1]. Before the introduction of vitamin D into milk in the Province of Quebec, the vast majority of cases of rickets treated at The Montreal Childrens' Hospital were the result of vitamin D deficiency; only a few cases of inherited rickets were seen. Following the addition of vitamin D to milk, only one case of vitamin-D-deficiency rickets was seen; thus the inherited forms of rickets came to represent the majority of rickets cases admitted, although the prevalence of these cases remained the same. In Scriver's words, "The rickets volume was greatly decreased but the heritability of rickets had increased greatly because the origin of rickets had shifted from an extrinsic cause (vitamin D deficiency) to intrinsic causes (Mendelian causes of phosphate homeostasis)." A similarly dramatic, but less well-documented change has occurred in the biologic origins of hematuria in North America and possibly in Europe. Poststreptococcal glomerulonephritis was, in the past, the most frequent cause of hematuria in North America. The incidence of poststreptococcal glomerulonephritis has decreased and relatively more patients are now being diagnosed with IgA nephropathy and with Alport's hereditary nephritis.

The investigation of a child with hematuria poses many difficulties, especially in deciding how many tests to do for microscopic hematuria. This is

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Table 6-1. Classification of causes of familial hematuria

Glomerular
Immune-mediated causes
Extrinsic
Acute poststreptococcal glomerulonephritis
Extrinsic–Intrinsic
Membranoproliferative glomerulonephritis
Systemic lupus
IgA nephropathy
Structural abnormalities of the GBM
Intrinsic
Alport's hereditary nephritis
Thin glomerular basement membrane
Nonglomerular
Extrinsic–Intrinsic
Idiopathic hypercalciuria
Intrinsic
Sickle-cell disease
Autosomal dominant polycystic kidney disease

especially true when the hematuria has been detected during a routine exam of an otherwise normal child. A phased approach to this problem has been described [2] and indications have been established for performing a renal biopsy [3]. The present chapter will deal with the modes of inheritance and the approach to genetic diagnosis and genetic counseling of a child and family with familial hematuria.

DEFINITION OF FAMILIAL HEMATURIA

Familial hematuria is defined as the occurrence of hematuria by history, or its detection by dipstick or microscopy in more than one member of a kindred. Familial is not synonymous with inherited because, for example, although more than one sibling may have poststreptococcal glomerulonephritis, there is as yet no persuasive evidence that this condition is genetically determined.

CLASSIFICATION OF FAMILIAL HEMATURIA

Conventional, comprehensive lists of the causes of hematuria in childhood do not classify hematuria on the basis of whether or not there is familial occurrence [2]. We propose an approach that takes into account the observation that hematuria often occurs in families (table 6-1). Accordingly, a cause of hematuria may be genetically determined (i.e., intrinsic) with no apparent extrinsic modifiers. Alport's hereditary nephritis syndrome and benign familial hematuria with thin glomerular basement membranes are example of intrinsic causes. Hematuria may occur as a result of the interaction of both intrinsic and extrinsic factors, for example in IgA nephropathy and in idiopathic hypercalciuria. Familial hematuria also may occur as the result of predominantly extrinsic factors, as in the case of acute poststreptococcal glomerulonephritis.

GLOMERULAR CAUSES OF HEMATURIA

Criteria for the diagnosis of a glomerular origin of hematuria are an association with at least one of the following findings: red blood cell casts, proteinuria, and dysmorphic red blood cells in the urine.

Immune-mediated causes of glomerular injury

Extrinsic (environmentally-induced hematuria)

Acute postinfectious (usually poststreptococcal) glomerulonephritis. Although poststreptococcal glomerulonephritis may occur in families with a high attack rate of 38% in sibling contacts, there is no known evidence of a genetic predisposition to nephritis after streptococcal infection [4].

Extrinsic–intrinsic

Membranoproliferative glomerulonephritis (MPGN). Conventionally, this group of conditions has not been thought of as having a heritable component. However, 29% of patients with types I and III MPGN have a major histocompatibility complex extended haplotype showing linkage disequilibrium on the short arm of chromosome 6 that is present in only 3% of normal individuals [5, 6]. This observation is not indicative of familial occurrence, but is associated with a poorer prognosis. Hematuria and/or proteinuria may be the initial presenting findings in 22% of patients with type I and 65% of those with type III MPGN [6].

Systemic lupus erythematosus (SLE). Although patients with lupus nephritis do not usually present with isolated hematuria in the absence of any other features of SLE, the evidence for a genetic contribution to the pathogenesis of SLE is so compelling that no discussion of familial hematuria would be complete without summarizing these data [7]. There is a greater concordance for SLE in monozygotic twins than in dizygotic twins, an association between SLE and genetically determined HLA-antigens (DR2, DR3), certain inherited deficiencies in the complement system (C2, C4), and defects of T-cell activity found in healthy family members of patients with SLE. These findings are compatible with multifactorial inheritance, in that extrinsic and intrinsic factors are causative when acting together. Because many factors are operative in the etiology of SLE, an empiric risk figure of 8% has been calculated for the occurrence of SLE for first-degree relatives.

IgA nephropathy. Hematuria is the clinical hallmark of IgA nephropathy. This may be recurrent and macroscopic, or persistent and microscopic. A definitive diagnosis can be made only by demonstrating the presence of IgA deposits in the glomerular mesangium by immunofluorescent microscopy, and by excluding other causes of mesangial IgA [8]. Many extrinsic causes of IgA nephropathy have been suggested, including cytomegalovirus [9]. There is also a growing body of evidence that suggests that genetic factors may also be important. This includes differences in the racial prevalence of the disease. Many patients (60%) studied in eastern Kentucky had affected relatives; about

40% of them occurred in one kindred [10]. In several studies, an association with certain HLA B or DR alleles was found. This did not occur in every kindred in which affected first-degree relatives had different HLA haplotypes [10]. Additional evidence for a genetic basis is the occurrence of the disease in identical twins. All of this information is suggestive of multifactorial inheritance, with a risk to a first degree relative of about 5%.

Structural abnormalities of the glomerular basement membrane (GBM)

Structure abnormalities of the GBM are by definition *intrinsic*.

Alport's hereditary nephritis

This hereditary nephropathy is characterized by recurrent or persistent microscopic hematuria, occasional gross hematuria, proteinuria, chronic renal failure that may be variable but is usually progressive (especially in affected males), and high-frequency sensorineural hearing loss. In some families, affected individuals may have ocular defects such as anterior lenticonus. The exact prevalence is unknown, but Hasstedt et al. (1983) have estimated a disease frequency of 1 in 5000 based on 300 affected people in a population of 1.5 million in Utah and southern Idaho [11].

Hematuria is the most frequent initial sign in children. It may be microscopic or, less frequently, macroscopic. Hematuria can be detected at or soon after birth in males and may persist into adulthood. Most females (85%) who carry the gene have hematuria [12] In our experience, the hematuria has often been mistakenly ascribed to poststreptococcal glomerulonephritis. When red blood cell casts are not seen, or if the patient does not have proteinuria, the diagnosis may be delayed or not even suspected unless a careful family history and urine examinations are obtained in every patient who presents with hematuria. Proteinuria does not tend to occur until about the second decade of life, and about 30% of the patients develop the nephrotic syndrome.

The usual difficulties faced by offering genetic counseling are compounded by the heterogenous nature of Alport's hereditary nephritis. This heterogeneity is exemplified by the inconstant association of the nephropathy with extrarenal anomalies as well as the variability of the occurrence, age of onset, and severity of renal involvement among kindreds. There is, however, less variability with regard to renal involvement within a kindred, especially in the same sex. Males not only manifest signs and symptoms earlier than females, but also are more severely affected. In some kindreds there is high-frequency sensorineural cochlear deafness. Affected individuals may be unaware of this deficit and therefore audiograms must be done (table 6-2). Some females may have a hearing deficit and normal urine. Other kindreds may have ocular defects (anterior lenticonus, cataracts, fundus albipunctus) as well as the deafness, while others may have them without hearing deficits. Some patients have an associated thrombocytopenia. In some kindreds there have been no extrarenal defects. The matter is further complicated by the fact that within a kindred there may be variable expression of the extrarenal manifestations. In addition

Table 6-2. Indications for formal audiometry in a patient with hematuria

1. A positive family history of hematuria
2. Hematuria with red blood cells casts with no obvious cause for the hematuria
3. Biopsy evidence of Alport's hereditary nephritis

Table 6-3. Classification of Alport's hereditary nephritis

Classification	Description
Type I	Juvenile nephritis with deafness. May or may not have ocular abnormalities. Dominant inheritance (? autosomal or X-linked).
Type II	Juvenile nephritis with deafness. May or may not have ocular abnormalities. X-linked dominant inheritance.
Type III	Adult-onset nephritis with deafness. No ocular abnormalities. X-linked dominant inheritance.
Type IV	Adult-onset nephritis. No deafness or ocular abnormalities. X-linked dominant.
Type V	Nephritis with deafness and thrombocytopathy. Autosomal dominant.
Type VI	Juvenile nephritis with deafness. Autosomal dominant.

to these difficulties, genetic counseling may be rendered even more fraught with difficulty because of the occurrence of apparently new mutations for Alport's hereditary nephritis in patients whose clinical and histopathologic features are indistinguishable from those with a positive family history of Alport's hereditary nephritis [13]. Fortunately, isolated microscopic hematuria, with a negative family history of hematuria in a first-degree relative and no episodes of macroscopic hematuria, is unlikely to be associated with abnormal renal biopsy findings [3].

Modes of inheritance of Alport's hereditary nephritis

The mode of inheritance of Alport's hereditary nephritis has not been elucidated perfectly and is a subject of much conjecture and controversy. The spontaneous mutation rate has been estimated at about 15% [14]. X-linked, autosomal dominant, and even autosomal recessive modes of inheritance have been proposed. Although it is possible that Alport's syndrome is genetically heterogenous, and that several modes of inheritance may occur, the X-linked seems to be the most likely [12].

Atkin et al. (1986) have proposed [14] that there may be six types of Alport's hereditary nephritis on the basis of apparent modes of inheritance, age of onset of end-stage renal failure in males (juvenile or adult), and extrarenal abnormalities (table 6-3). Within a kindred, the ages at which affected males entered end-stage renal failure tended to be similar in that they were within about five years of the intrakindred mean age [14].

X-linked inheritance is suggested in many kindreds by the transmission of

the disorder from one generation to the next, from mother to son or daughter, and from father to daughters only. (When the gene is on the X-chromosome it cannot be passed from father to son because the son receives the Y-chromosome from his father.) In the 23 Utah kindreds ascertained by Hasstedt et al. (1986), there were no male-to-male transmissions [12]. However, these data may be biased by the fact that many affected men are in end-stage renal failure at an early age and consequently may not have offspring. With the advent of the technique of recombinant DNA, the specific locus or loci for Alport's hereditary nephritis in the genome will be mapped, and the mode(s) of inheritance will be revealed. Three large Utah kindreds with a total of 259 members were studied with five DNA markers for the X-chromosome [15]; these showed definite evidence of linkage of Alport's syndrome to the long arm of the X-chromosome. One of these kindreds, P, type III, had been described originally by others as being partially X-linked [16]. The other two kindreds were classified as type IV. Perhaps the same gene is affected in both types.

With X-linked transmission, each son and daughter of an affected mother has a 50% risk of inheriting the gene. The daughter of an affected father has a 100% chance of inheriting the gene. In fact, however, 82% of all female carriers showed signs of the disease. In other words, 82% penetrance for the adult type and 28% for the juvenile type is indicated [12, 17]. Other studies have suggested autosomal dominant inheritance with abnormal segregation [18, 19]. These results may have been partially the consequence of incomplete ascertainment due to the lack of follow-up studies and the absence of audiograms and eye examinations.

Hematuria associated with thin glomerular basement membrane

The diagnosis of benign familial hematuria is made by exclusion of proteinuria, progressive renal insufficiency, hearing deficits, or ophthalmologic abnormalities in the patient and family members. The hematuria is usually microscopic, the red blood cells tend to be dysmorphic, and red blood cell casts may be seen. Macroscopic hematuria may occur in association with an upper respiratory tract infection. The primary pathologic changes are thinning of the glomerular basement membrane. Variable degrees of lamination of the glomerular basement membrane may cast doubt on the diagnosis and raise the specter of Alport's syndrome [20]. The inheritance of benign familial hematuria may be autosomal dominant [21] or autosomal recessive [22].

NONGLOMERULAR CAUSES OF HEMATURIA

Extrinsic–intrinsic

Idiopathic hypercalciuria

Hypercalciuria, with or without renal calculi, is one of the most common putative causes of microscopic and/or macroscopic hematuria in children [23]. Males are affected more often than females, and whites more often than blacks.

Hypercalciuria and/or stone formation appear to be inherited by an autosomal dominant mode with variable penetrance [24] and possible variable expression because stones may develop in some family members without hypercalciuria [25].

Intrinsic

Sickle-cell disease

Hematuria is often seen in patients with sickle-cell disease and in those with sickle-cell trait. This makes it different from most autosomal recessive conditions, where only the homozygote is affected. The hematuria occurs in a carrier parent or sibling (heterozygote) who does not have any other signs of the disease.

Autosomal dominant polycystic kidney disease

Patients with this condition may present with hematuria, but diagnosis is not usually a major problem now that renal ultrasonography, CT scanning, and DNA probes have become available.

APPROACHES TO GENETIC DIAGNOSIS AND COUNSELING OF PATIENTS AND THEIR FAMILIES WITH FAMILIAL HEMATURIA

1. Not all causes of familial hematuria have a proven genetic basis, e.g., poststreptococcal glomerulonephritis.
2. Not all causes of hematuria that have a genetic basis require genetic counseling *for the hematuria per se*, e.g., hematuria associated with Sickle-cell disease or sickle-cell trait.
3. The penetrance and expression of the gene may vary from family to family and within families. There is no pathognomonic feature of Alport's hereditary nephritis. The so-called characteristic ultrastructural lesion in the glomerular basement membrane may develop during the course of the disease and may not be present in every affected member of a kindred [26]. There is still considerable controversy concerning the precise mode or modes of inheritance of the Alport's gene or genes.
4. Although the prognosis of familial hematuria associated with thin glomerular basement membranes appears to be excellent, it is not always possible to differentiate with absolute assurance between this entity and Alport's syndrome [20].
5. The importance of offering genetic counseling will depend on a number of factors, which differ from family to family.
6. A patient with an inherited cause of hematuria (e.g., sickle-cell disease) may have a superimposed acquired cause of hematuria (e.g., acute poststreptococcal glomerulonephritis).
7. Specific genetic probes are not yet available for the precise diagnosis of Alport's syndrome.

8. There may or may not be an overlap between Alport's syndrome, with its potentially poor prognosis, and benign familial hematuria, with its apparently excellent prognosis.

DIFFICULTIES OF GENETIC COUNSELING IN ALPORT'S HEREDITARY NEPHRITIS: TWO EXAMPLES

Family F. During childhood a boy had several episodes of what was diagnosed as acute poststreptococcal glomerulonephritis. Difficulties with hearing became manifest during early adolescence. By mid-adolescence he developed end-stage renal failure and received a renal allograft. The typical basket-weave pattern of the glomerular basement membrane of Alport's hereditary nephritis was seen on ultrastructural examination of his kidney. He is now 35 years of age and has not fathered any children. His two adult sisters do not have hematuria and have normal audiograms. Routine blood tests were done on his mother (at age 55 years) prior to a cholecystectomy, and these showed that she was in chronic renal failure. The features of Alport's hereditary nephritis were found in her glomeruli, and previously unrecognized high-frequency sensorineural hearing loss was diagnosed by an audiogram.

Comment: This family demonstrates probable X-linked dominant inheritance of juvenile Alport's syndrome. And yet, autosomal dominant inheritance cannot be excluded. The son was affected earlier and more severely than his mother.

Counseling would be offered as follows: The risk for each of the proband's sons (if he were to father any) would be negligible if one takes into account a low probability for autosomal dominant inheritance. Each of his daughters would have a 50% risk of inheriting the gene and a 28% chance of manifesting the renal disease (penetrance). Thus, the risk of a daughter inheriting the gene and manifesting the nephritis would be 14% (50% × 28%). The proband's sisters would have had a 50% chance of inheriting the gene and a 72% chance of not manifesting any signs of the nephritis (nonpenetrance). Their actual risk of having the gene is calculated at 36% (50% × 72%). If X-linked inheritance is assumed to have occurred in this family, then each of the daughter's own daughters would have a 5% (14% × 36%) chance of inheriting the gene and of showing signs of renal involvement.

Family S. At age three months, this boy had hemophilus meningitis. During his illness, which was severe, microscopic hematuria was detected by dipstick on many occasions. The attending pediatrician, who also happened to be a nephrologist, chose not to pursue the cause of the hematuria, ascribing it vaguely to the bacterial infection. At age three years, the mother noticed that her son was having difficulty hearing (she had been told that this might be a sequel of the meningitis). An audiogram showed high-frequency, sensorineural deafness. Examination of his urine sediment revealed numerous red blood cells and red blood cell casts. Alport's here-

ditary nephritis was confirmed by the appearances seen on a renal biopsy specimen. His mother had repeatedly denied a family history of familial nephritis, but admitted only after she was found to have microscopic hematuria with red blood cell casts that her brother had died of chronic renal failure at age 16 years.

Comment: This family illustrates how difficult it can be to make a diagnosis of Alport's hereditary nephritis when the history is confounded by another disease, when the age of presentation seems to be atypical, and when the family members are unable to answer very painful questions. The probable mode of inheritance appears to be juvenile X-linked dominant. Genetic counseling is similar to that offered to family F.

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7. GENETICS OF CONGENITAL AND EARLY INFANTILE NEPHROTIC SYNDROMES

OLLI KOSKIMIES

The congenital nephrotic syndrome (CoNS) is characterized by the presence of proteinuria at birth. Marked glomerular proteinuria leads to protein depletion, a low serum protein level, edema, dyslipidemia, and disturbances in fluid and electrolyte homeostasis. Overt nephrotic syndrome develops; the infant fails to thrive, and her/his psychomotor development is disturbed. It was thought that the age of three months separates congenital from infantile forms of NS. This division proved to be arbitrary. The diagnosis has to be based on clinical, laboratory, and histological criteria, rather than age of onset.

NS detected soon after birth can be acquired, idiopathic, or part of another syndrome [1] (table 7-1). The acquired CoNS is most often due to syphilis [2], but it can also be caused by intrauterine toxoplasmosis, rubella, or cytomegalovirus infection [3–5]. Renal venous thrombosis has rarely been the cause of CoNS [6]; usually thrombotic events represent complications of the nephrotic state. Other diseases with edema, e.g., Turner's syndrome and erythroblastosis fetalis, are easily differentiated from NS based on their specific clinical features and the lack of continuous heavy proteinuria.

This chapter will concentrate on those types of congenital and early infantile NS that are known or considered to be genetically determined.

CONGENITAL NEPHROTIC SYNDROME OF THE FINNISH TYPE (CoNSF)

Among the various types of nephrotic syndromes occurring in the newborn infant, CoNSF is the best characterized. This is a recessively inherited auto-

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Table 7-1. Classification of congenital and early infantile nephrotic syndromes

Acquired types
Congenital syphilis
Other perinatal infections
Renal vein thrombosis
Idiopathic types
Congenital nephrotic syndrome of the Finnish type
Diffuse mesangial sclerosis
Other glomerular diseases
Syndromic congenital nephrotic syndromes
Nephropathy associated with brain malformations
Drash syndrome
Nail-patella syndrome

somal disease most often seen among people of Finnish origin [7]. The CoNSF gene frequency for Finland has been calculated to be 1:200. Usually the infant is born prematurely and is small for date. The placenta is invariably large, over 25% of the newborn's birth weight. The infant often has widely open cranial sutures, due to delayed ossification, and a small, low-bridged nose. Asphyxia and respiratory distress are common [8, 9]. Proteinuria, which is already present in the second trimester of pregnancy, soon results in overt NS with typical biochemical alterations. During the early stages of the disease proteinuria is selective [10, 11]; later on, erythrocytes, leukocytes, amino acids and glucose can also be present in the urine [9]. Renal histological findings are not pathognomonic, but dilated proximal tubules as well as mesangial accentuation and glomerular hypercellularity can be demonstrated in most cases. As the disease progresses, cellular infiltration and fibrosis involve the interstitium and surround the glomeruli [9, 12].

The CoNSF is due most likely to a defect of the glomerular basement membrane [13]. So far, no treatment has been shown to be effective in preventing the protein leakage. If left without supportive treatment, the infant fails to thrive, develops gross edema, and usually dies due to septic or thrombotic complications. The clinical course can be modified by aggressive nutritional support and careful control of the tendency towards edema and infections. When conservative management no longer sustains the growth and development of the child, renal transplantation should be considered [14].

CoNSF has an autosomal recessive mode of inheritance. Norio [7] performed a genetic study of 57 Finnish families and found the proportion of affected sibs to be very close to 0.25 and the sex ratio to be 1.07. Consanguinity of the parents was present in 16 families. The ancestry originated from a large geographic area that has been populated permanently for less than 500 years. In fact, CoNSF was the first of the rare recessive disorders discovered to be overrepresented among the Finnish people. The heterozygotes for the CoNSF are healthy and cannot be recognized by any known biochemical method [16].

A definite diagnosis is necessary for genetic counseling. In addition to the NS, the following features have invariably been present in CoNSF: large placenta, selective proteinuria at birth, and signs of retarded skull ossification. Renal insufficiency was never observed during the first months of life. If any of these signs is missing, a form of NS other than CoNSF has to be considered. Renal biopsy findings may help to establish the diagnosis.

CoNSF is already manifest during the second trimester of pregnancy and it can be reliably diagnosed at the 15th to 18th week of gestation by measuring the alpha-fetoprotein (AFP) in the amniotic fluid. High AFP levels in the amniotic fluid in a mother who already has one child with CoNSF is very suggestive of CoNSF in the fetus [17]. Thus, a family with an index child should be informed of the possibility of prenatal diagnosis, and of having the pregnancy interrupted in case of fetal disease. Most high-risk families in Finland have accepted this alternative.

Increased levels of AFP in maternal serum could also disclose the existance of CoNSF [17, 18]. Screening based on maternal serum AFP concentration was initiated in 1978 in an area of Finland where the incidence of CoNSF is high. The diagnosis of CoNSF was verified by amniocenthesis and proven by anatomopathologic investigation of the legally aborted fetuses. The concordance among these tests was excellent [18].

If the infant's clinical manifestations are not those of CoNSF, estimation of the risk of recurrence in successive pregnancies is difficult. Unfortunately, reliable methods of prenatal diagnosis are not available in the majority of other types of CoNS.

NEPHROTIC SYNDROME WITH DIFFUSE MESANGIAL SCLEROSIS (DMS)

In 1973, Habib and Bois [19] described the clinicopathological features of a series of 37 infants with NS. The authors divided the histological findings into three main categories; those typical of CoNSF, those of minimal change or focal glomerular lesions, and a form they designated as diffuse mesangial sclerosis (DMS). Since 1973, several investigators have confirmed the existence of NS with DMS lesions in glomeruli, sometimes present already at birth, sometimes appearing in infancy or early childhood.

In those publications in which the neonatal history has been recorded, the children were described to have normal birthweights, normal plancentas, and uneventful immediate postnatal courses [9, 20]. The disease is refractory to all treatment, and the patients have been reported to die because of renal failure rather than infection or fluid imbalance, as is the case in infants with CoNSF [9, 19–21].

Histologically, the glomerular capillary tuft is contracted, devoid of patent capillaries, and often lined with a layer of epithelial cells; the urinary space is dilated [9, 19]. It has been suggested that DMS is the histologic expression of more than one clinical entity [22]. Indeed, patients with and without various malformations have been reported to share this glomerular lesion. Two sibl-

ings with DMS histology were described to have ocular abnormalities [21]. At least some of the patients with the typical Drash syndrome have a renal histology compatible with DMS [22]. An infant with a nephropathy associated with brain malformations, followed in our hospital, was found at postmortem examination to have renal changes similar to those of DMS (H. Sariola, J. Rapola, personal communication).

There is an increased familial incidence of DMS. All patients have been found to appear in one generation. In the original description of DMS, two families were reported, one with three affected and four healthy siblings, and the other with three affected (including a pair of monozygous twins) and two healthy siblings [19]. At least three other families have been described to have two or more children affected by this disease [20,21]; in one of these families there was consanguinity between the parents [20]. We have followed two siblings with biopsy-proven DMS. A newborn girl had slight edema and later, at the age of five months, developed overt NS. She died at the age of ten months of chronic renal failure. Her brother presented with hypertension and chronic renal failure as late as five years of age; two years later he received a renal transplant and is now 11 years old, a normally grown and developed schoolboy. He has moderately elevated serum creatinine. The family includes two older children who are healthy.

Although a systematic study of the mode of inheritance of DMS has not been possible because of the rarity of this nephropathy, familial occurrence and consanguinity [20] indicate that DMS is an autosomal recessive disease, like CoNSF. Schneller et al. [23] reported normal amniotic fluid AFP levels in association with a fetus who had CoNS and renal histologic changes compatible with DMS. Other attempts at prenatal diagnosis have not been reported. It is possible that in this disease there is no intrauterine proteinuria. If this is the case, intrauterine diagnosis will be impossible until the gene defect, if any exists, is identified.

NEPHROPATHY ASSOCIATED WITH BRAIN MALFORMATION

Galloway and Mowat [24] were the first to report two siblings with congenital microcephaly, hiatus hernia, and NS. A second family has been reported in which two of three children, a female and a male, had the same triad. There was no consanguinity, and the parents were healthy [25]. A male infant, followed in our hospital, had dysmorphic features of the face, cerebral and cerebellar malformations, and developed NS during the first week of life. He was the second child, born after an uneventful pregnancy, and small for date; the size of placenta was normal. The first child of the family was a healthy girl. The patient died at the age of two months of undefinable causes. The kidneys showed diffuse mesangial sclerotic changes in the glomerular mesangium and cystic dilatation of the renal tubules.

Palm and coworkers [26] reported in 1986 on two male siblings with a combination of brain malformations and nephropathy. The older one had

proteinuria, documented at three weeks of age, and developed NS at the age of five months. He died at the age of two years and ten months. In a subsequent pregnancy, the AFP concentration in the amnoitic fluid was found to be increased 20-fold at 17 weeks of gestation, and the pregnancy was terminated. The kidneys had several small cysts located mainly at the corticomedullary junction. Electron microscopy disclosed slight glomerular changes, consisting of fused podocytes and irregularities of the basement membranes. The central nervous system findings were similar to those of the older brother. There were two healthy children in the family, and the parents were not consanguinous.

It may be that these rare cases represent different syndromes or different degrees of severity within the same syndrome. In the latter family, the pattern of inheritance could be either X-linked or autosomal recessive; earlier reports have suggested the microcephalus–hiatus hernia–nephropathy triad to be inherited as an autosomal recessive trait [24, 25]. AFP determination in amniotic fluid proved to be of diagnostic value in one case [26].

OTHER TYPES OF CONGENITAL AND INFANTILE NEPHROTIC SYNDROMES

The occurrence of male pseudohermaphroditism (XY gonadal dysgenesis), glomerulopathy, and Wilms' tumor has been known for about 20 years [27]. This triad is referred to as Drash syndrome [28]. The renal disease presented as CoNS in 14% of the patients, infantile NS in 41%, and proteinuria between one and three years of age in 27% of patients [29]. Chronic renal failure developed usually within months after birth, often before the Wilms' tumor was diagnosed [22, 29]. The renal histological findings of Drash syndrome are similar to those of DMS with the addition of tubular atrophy [22]. We have treated an infant with Drash syndrome who had a large placenta, NS from birth, and who developed chronic renal failure within the first two weeks of life. The diagnosis of DMS was confirmed at autopsy [1].

All cases of Drash syndrome have been sporadic, except for a pair of monozygous twins. It is unknown what disturbance of early embryogenesis causes this triad of urogenital malformations. The risk of recurrence in a family appears to be very small, and there is no evident pattern of inheritance. It is of interest that the DMS histology, or *NS without Drash triad*, may be inherited in a recessive fashion, while DMS in association with Drash syndrome occurs sporadically. Thus, the relevance of DMS has to be interpreted in the context of a complete investigation that should include chromosome analysis [1].

An occasional patient with clinically and genetically proven, dominantly inherited nail-patella syndrome may present as a CoNS [30]. One of these patients had aplasia of the patellae and a limitation in extension at the elbows. The placenta was normal. NS disappeared spontaneously. Several patients with congenital or early infantile NS with minimal or focal glomerular changes on renal biopsy have been described [19]. The outcome of these infants seems to

Table 7-2. Clinical and genetic differences between various types of congenital and early infantile NS

	CoNSF	DMS	Syndromic forms of CoNS
Genetics	Autosomal recessive	Familial cases common	Variable, familial cases reported
Placenta	Large, 25% birth weight	Normal	Normal (occasionally large)
Perinatal findings	Prematurity with associated problems	Not specifics	Variable
Onset of proteinuria	2nd trimester of pregnancy (selective proteinuria)	Variable, later than CoNSF	Variable
Associated malformations	None	Only occasionally	Always
Clinical features	If aggressively treated patients grow and develop. serum creatinine normal	Growth variable, renal failure develops	Growth variable, psychomotor retardation common, renal failure develops
Cause of death	Infections, thrombotic complications	Renal failure	Renal failure, sudden death (Wilms' tumor)
Prenatal diagnosis	Elevated AFP in amniotic fluid and usually in maternal serum	Not available, AFP normal	Not available (amniotic fluid AFP occasionally elevated)

CoNSF: congenital nephrotic syndrome of the Finnish type
DMS: diffuse mesangial sclerosis
AFP: alpha-fetoprotein

be variable. In general, however, they seem to do better than patients with CoNSF. Larbre [31] described a child with CoNS who at the age of 12 years was still nephrotic and growing rather poorly but was not uremic. These types of NS may be a result of polygenic patterns of inheritance, as appears to be the case with the idiopathic nephrotic syndrome of childhood [32]. An increased frequency of HLA-B12 and HLA-A1/B8 has been described in several series of patients with minimal-change NS and atopy, and a strong association of NS with HLA-DR7 has also been documented [33]. The possible relationship between these genetic markers and the inheritance of NS in general, and of the infantile forms in particular, is yet to be determined.

CONCLUSIONS

CoNSF is a clearly defined clinical entity, characterized by large placenta, normal renal function, and proteinuria, always present during the intrauterine life (table 7-2). The diagnosis can be confirmed by renal biopsy. Because CoNSF is an autosomal recessive disease, parents of an index infant have to be informed that prenatal diagnosis is possible.

A diagnosis can also be made in most of the patients with other types of CoNS. Clinical and histopathological findings have to be considered in concert. Familial cases are common; DMS may be inherited recessively. In general, prenatal diagnosis is not available for this group of diseases, perhaps because these diseases manifest first at or after birth.

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III. TUBULAR DISORDERS

8. HEREDITARY TUBULAR TRANSPORT ABNORMALITIES

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In this chapter we discuss a group of human mutations that affect renal tubular transport processes. Since the list is lengthy, we have tried to cluster disorders according to certain functional characteristics. For each category we have chosen one disorder as a prototype, while the others are discussed only briefly or in tabular form.

We first consider mutations affecting specific organic solute transport systems in the proximal tubule, such as the 15–20 discrete transport systems supporting the reabsorption of specific amino acids and glucose. Cystinuria is discussed as the prototype. These disorders rarely deplete the patient's body stores, but have a specific urinary phenotype.

The second group consists of hereditary disorders of proximal renal tubular reabsorption, which produce systemic depletion of specific inorganic solutes. Hypophosphatemic rickets will be discussed briefly as a representative of this group.

The third category includes various hereditary forms of distal renal tubular acidosis.

Several specific reabsorptive processes in the renal tubule are highly regulated by hormones; disturbances in hormonal control form the fourth group covered in this chapter. Nephrogenic diabetes insipidus will be presented in detail as a prototype of this category of diseases.

The fifth and last group consists of several hereditary multisystem diseases affecting a wide range of reabsorptive processes in the proximal tubule, i.e., the renal Fanconi syndrome. Cystinosis is the prototype.

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MUTATIONS AFFECTING PROXIMAL TUBULAR REABSORPTION OF ORGANIC SOLUTES WITHOUT PRODUCING SYSTEMIC DEPLETION: SPECIFIC AMINOACIDURIAS AND GLUCOSURIA

A number of biologically useful solutes such as amino acids and glucose are in the proximal convoluted tubule. Although most of the individual transport proteins have not been isolated and characterized biochemically, spontaneous human mutation and in vitro studies provide ample evidence that the proximal tubular brush border membrane contains at least 15–20 discrete transport systems to support the reabsorption of amino acids and glucose. Individual mutations may affect the transport of specific subgroups of amino acids: 1) dibasic amino acids (lysine, arginine, ornithine); 2) cystine plus dibasic amino acids; 3) neutral amino acids excluding glycine, proline, and hydroxyproline; 4) neutral amino acids including sarcosine, glycine, proline, and hydroxyproline; 5) anionic amino acids (glutamate and aspartate); and 6) beta-amino acids (taurine and beta-alanine) [1]. It is likely that each of these amino acid subgroups is served by a specific carrier mechanism that has a relatively high maximal transport capacity capable of bulk reabsorption from the proximal tubular fluid. As the concentration of amino acids falls during passage along the tubule, a second class of transport mechanisms assumes increasing importance. These carriers tend to be more selective and are more efficient at low substrate concentrations. Although the transport systems have not been precisely localized, there is some evidence that these two classes of carriers are arranged in sequence along the nephron [2, 3]. Thus, reabsorption of amino acids and glucose by low-affinity high-capacity systems appears to be followed by reabsorption via high-affinity low-capacity systems, which mop up the remaining amounts of each substrate. Final excretion of these substances is generally 5% or less of the filtered load [1].

Mutations that perturb these discrete transport processes are easily identified because they result in hyperexcretion of amino acids or glucose in characteristic patterns. However, they do not usually produce a depletion syndrome. In part, this is due to the redundancy of transporters described above; alternate mechanisms always allow partial conservation of the substrate. Secondly, the blood levels of these organic substrates are buffered by large metabolic pools. However, under special circumstances defective transport of these substrates may produce enough depletion to be of medical importance.

Cystinuria

Cystinuria is an autosomal recessive disease affecting reabsorptive transport of cystine and dibasic amino acids (ornithine, arginine, and lysine) by the brush border membrane of the kidney and small intestine. The condition is easily diagnosed by the presence of hexagonal cystine crystals in urine or by the cyanide–nitroprusside urine test, which detects elevated amounts of cystine (more than 100 mg/g creatinine). The reported incidence of cystinuria varies according to geographic region and method of ascertainment. Levy [4] sum-

marized the data obtained by screening programs, and concluded that the overall prevalence is about 1 in 7000 live births. This is in agreement with an adjusted estimate in the Quebec population made by Scriver et al. [5].

As mentioned above, cystinuria is not a depletion syndrome; plasma amino acid levels are within the normal range [1]. The primary clinical consequence is the formation of cystine stones within the urinary tract, often causing obstruction, infection, or renal failure. Because of the low solubility of cystine in acid urine, patients who excrete more than 1–1.5 mmoles of cystine per gram creatinine are likely to form stones. Although stone formation by homozygotes has occurred as early as the first year of life, most patients do not have clinical evidence of urolithiasis before the end of the second decade [6]. Among the factors known to favor the crystallization of cystine are high urine pH and low urine volume [7]. It is not clear, however, why a few percent of homozygotes form stones at an early age while most of them are protected until the third decade [8].

Harris et al. [9] recognized that parents of stone-forming cystinuric patients could be completely recessive heterozygotes with normal excretion of cystine or they could be incompletely recessive with moderate hyperexcretion. Studies by Rosenberg et al. [10] suggest that this heterogeneity is explained by the presence of at least three different mutations at the cystinuria locus in human populations. In cystinuria type I, the homozygote excretes relatively large amounts of cystine, lysine, arginine, and ornithine in the urine. Heterozygotes have no abnormal aminoaciduria and are silent carriers. On the basis of a study made in Brazil, Guigliani et al. [11] concluded that there is a high frequency of heterozygotes for types II and III cystinuria among urinary stone-formers, indicating that heterozygosity for these genes is a risk factor for urinary stone formation. Twenty-five percent of the progeny of two heterozygous individuals will be homozygous (e.g., II/II) or genetic compounds (e.g., II/III). Both these types will have cystinuria, but the various combinations of mutant alleles may explain in part the variation in clinical severity.

Diagnosis of classical cystinuria is based on several criteria. Firstly, aminoaciduria must be confined to cystine and the dibasic amino acids; a broader dysfunction of the proximal tubule (renal Fanconi syndrome) must be ruled out. Secondly, the disease must be distinguished from mutations at different transport loci: 1) hyperdibasic aciduria type I is an autosomal recessive disorder of the brush-boarder-membrane dibasic-amino-acid transporter that does not affect cystine transport; 2) hyperdibasic aciduria type II (lysinuria-protein intolerance) is an autosomal recessive disease prevalent among Finns in which the renal and intestinal basolateral membrane carrier for dibasic amino acids is defective; 3) isolated hypercystinuria (described in only one family thus far) is a disorder of the renal tubular transport system for cystine that does not affect dibasic amino acids transport. A diagnostic pitfall may occur in screening programs based on measurements of amino acid excretion during the first month of life. Scriver et al. [5] pointed out that the apparent prevalence of

Table 8-1. The features of benign familial glucosuria and eight distinct aminoacidurias

Disorder	Clinical findings	Pathophysiology	Genetics	References
Cystinuria	See Text	See Text	Autosomal recessive	1–11
Dicarboxylic aciduria	Glutamic-aspartic aciduria Plama levels normal or slightly decreased Apparently benign but 1 case with fasting hypoglycemia	Uncertain Transport defect in proximal tubule and intestine suspected, but could be an error in metabolism of these amino acids	Autosomal recessive Incidence 1:50,000 Heterozygotes have normal urine amino acids	12–13
Glucosuria (benign familial)	Glucosuria (0.5mM/L) when plasma glucose is 6mM/L Benign except in premature infants where caloric loss may compromise growth Daily glucose excretion of 2–30 g/day in adults	Reduced proximal tubular threshold for glucose	Evidence for existence of subtype now controversial Autosomal dominant or autosomal incomplete recessive Linkage to HLA complex Incidence 1:20,000	14–16
Hartnup disorder	Selective neutral aminoaciduria Usually benign, but in 10% pellagra-like photosensitive rash, intermittent behavioral manifestations, and ataxia may appear when essential amino acid and nicotinic acid nutrition is marginal Plasma amino acid levels are slightly reduced but usually within normal range	Defective transport of neutral aliphatic and ring-structured amino acids in proximal brush border of renal tubule and small intestine Unabsorbed tryptophan in gut is degraded to indoles by bacteria, absorbed and excreted in urine Tryptophan deficit may occasionally compromise nicotinic acid synthesis	Autosomal recessive; genetic heterogeneity is likely Incidence 1:15,000 to 1:100,000 depending on ethnic background Heterozygotes have normal amino acid excretion Maternal Hartnup disorder probably benign to the fetus	17
Hypercystinuria	Hyperexcretion of cystine but not ornithine, arginine, or lysine Benign except for probable risk of cystine calculus formation One family also has idiopathic hypoparathyroidism	Presumed defect in proximal tubular transport mechanism selective for cystine	Presumed autosomal recessive but only one family reported	1,116

Table 8-1 (continued)

Disorder	Clinical findings	Pathophysiology	Genetics	References
Hyperdibasic amino-aciduria type I	Hyperexcretion of lysine, arginine, ornithine Developmental delay in some patients (?homozygotes)	Defective transport system shared by dibasic amino acids probably located in brush border membrane of proximal tubule (and perhaps small intestine)	Autosomal dominant or autosomal incomplete recessive Heterozygotes have dibasic aciduria but are clinically well Incidence unknown	18, 19
Hyperdibasic amino-aciduria type II (lysinuria-protein intolerance)	Hyperexcretion of lysine, arginine, ornithine Failure to thrive Protein aversion Hepato-splenomegaly Episodes of vomiting, stupor, and hyperammonemia after excessive protein intake Mental retardation may occur Cystine excretion is normal Low plasma levels of dibasic amino acids	Defective transport system shared by dibasic amino acids probably expressed in basolateral membrane of proximal tubule, intestine and possibly liver Depletion of arginine and ornithine pools may occur, interfering with urea cycle function. Citrulline supplementation primes urea cycle and reduces hyper-ammonemia, orotic aciduria	Autosomal recessive Incidence 1:60,000 in Finalnd, lower elsewhere Heterozygotes are unaffected	20, 21
Hyper-histidinuria	Hyperexcretion of histidine with normal levels in plasma Mental retardation Myoclonic seizures	Defect in proximal tubular transport system selective for histidine	Only 4 cases reported (2 sibs) Presumed autosomal recessive	22
Iminoglycinuria	Hyperexcretion of amino acids (proline, hydroxy-proline, glycine) Probably benign Normal plasma levels of amino acids	Defect in proximal tubular transport system shared by glycine and amino acids, presumably expressed in brush border membrane	Autosomal recessive Heterozygotes may have modest glycinuria or may be normal, suggesting two different mutant genotypes in the population	117, 118

cystinuria in Quebec neonates (1–2 months) was seven times the estimated frequency in the older population. Longitudinal studies showed that in 75% of these infants, cystine excretion fell into the heterozygous range by the age of six months [5]. Thus, the transport mechanism appears to undergo late postnatal maturation; neonates with one mutant and one normal but immature allele appear to be homozygotes (or genetic compounds). Final classification should not be made until 6–9 months of age.

Glucosuria and other amino acidurias

The features of benign familial glucosuria and seven distinct aminoacidurias are outlined in table 8-1 [12–22]. Like the prototype for this group (cystinuria), each of these disorders has a unique urinary phenotype due to defective proximal tubular transport of a specific class of organic solutes. As discussed above, mutations that alter transport systems of the renal and intestinal brush border membrane do not usually produce systemic depletion syndromes. Thus, the clinical importance of recognizing benign familial glucosuria is to distinguish it from diabetes mellitus [14–16]. Medical complications of cystinuria (and presumably isolated hypercystinuria) arise from the low solubility of cystine. On the other hand, the Hartnup disorder is a good example of a mutation that is ordinarily benign but that may be significantly disadaptive when other genetic and environmental circumstances compromise the tryptophan pool [17]. Hyperdibasic amino aciduria type II (lysinuria-protein intolerance) typically produces failure to thrive, episodes of obtundation, and hyperammonemia from depletion of urea cycle intermediates [20, 21]. Unlike some of the other aminoacidurias, this defect is expressed in the renal tubule basolateral membrane and in a variety of other tissues including fibroblasts [20]. The basis for mental retardation and myoclonic seizures in histidinuria is unclear, since plasma levels of histidine are normal [22].

HEREDITARY DISORDERS OF THE RENAL TUBULE PRODUCING SYSTEMIC DEPLETION OF SPECIFIC INORGANIC SOLUTES

There is a variety of hereditary disorders affecting renal tubular transport of specific inorganic solutes such as phosphate, bicarbonate, potassium, or magnesium. In general, these mutations result in systemic depletion syndromes. The central principle of therapy involves replenishment of body stores by chronic oral supplementation of the relevant solute. X-linked hypophosphatemic rickets will be discussed briefly as the prototype of this group of diseases, although it is covered in detail in a later chapter.

X-linked hypophosphatemic rickets

X-linked hypophosphatemic rickets (XLH) is a disorder of the parathyroid-sensitive sodium-dependent mechanism for phosphate reabsorption in the proximal tubule [23]. Normally, 85%–95% of filtered phosphate is reabsorbed (about 60% in the proximal convoluted tubule, 15%–20% in the

straight proximal tubule, and 5%–10% at distal sites). Patients with XLH reabsorb only 40%–60% of filtered phosphate and develop hypophosphatemia (1–3 mM). A second feature is the failure of the renal tubule to respond to hypophosphatemia or parathyroid hormone infusion with an appropriate increase in the production of 1,25(OH)2-vitamin D; basal levels are often low or inappropriate, although in the normal range [24]. The combination of hypophosphatemia and dysregulation of renal vitamin D metabolism lead to linear growth failure and skeletal deformities due to osteomalaca.

Affected males may have normal levels of serum phosphate during the first few months of life, presumably due to the relatively low GFR prevailing at this age. Serum alkaline phosphatase levels begin to rise by 4–6 months, and linear growth failure is usually evident by the end of the first year. At presentation, serum calcium is normal, serum phosphate is low, serum alkaline phosphatase is markedly elevated, and the tubular reabsorption of phosphate is depressed (TRP=30%–60% vs normal=85%–95%; TmP/GFR=1–3 vs normal=4.5–8.0). Urinary calcium excretion is normal. Female hemizygotes are usually fully affected but tend to have slightly milder expression of the disease than their male counterparts. Bones seldom fracture but defective mineralization of metaphyseal growth regions produces progressive bowing of weight-bearing long bones. In childhood, defective dentine leads to frequent dental abcesses. Aside from the marked skeletal deformities, untreated adults are relatively asymptomatic, except that they may report muscle weakness and there is ectopic calcification at sites of tendon insertion [25]. The indication for therapy in adults is controversial.

At the present time, therapy of XLH is not entirely satisfactory. Healing of the rickets, improvement in growth velocity, and partial remodeling of skeletal deformities can be achieved by therapy with oral phosphate (1–2 grams divided into 4–5 doses per day) and vitamin D (e.g., 20–30 micrograms/kg/day of 1,25(OH)2-vitamin D). Longitudinal monitoring is required to ensure appropriate adjustment of therapy [26]. Excessive vitamin D therapy may produce episodes of hypercalciuria, hypercalcemia, and nephrocalcinosis [27]. Protracted excessive phosphate therapy may account for the appearance of tertiary hyperparathyroidism during the second or third decade in some patients [28].

Using linked chromosomal markers, the classic type of X-linked hypophosphatemic rickets has been mapped to the distal short arm of the X chromosome [29]. In addition, there is clinical evidence of autosomal mutations leading to additional forms of hypophosphatemic rickets [30–32]. These genetic considerations are discussed in detail in the next chapter.

Other tubular transport defects leading to depletion of inorganic solutes

Two familial syndromes of renal magnesium wasting have been described. A cluster of case reports recently reviewed by Rodriguez-Soriano [33] suggests that there may be an autosomal dominant form of isolated magnesium wasting

that is associated with hypomagnesemia, muscle weakness, and abnormal behavior. A second syndrome of magnesium wasting is apparently autosomal recessive and is associated with hypercalciuria, nephrocalcinosis, and mild distal renal tubular acidosis [33, 34]. These disorders have not been firmly established as distinct genetic entities.

At least two variants of the Bartter syndrome have been reported. In the classic form, failure to thrive and developmental delay are associated with hypokalemic alkalosis, renal salt wasting, stimulation of renin and aldosterone production, hyperexcretion of prostaglandins, and normal blood pressure [35]. Most patients come to medical attention within the first year of life, and many have a distinctive facies. If untreated, these children are chronically ill with recurrent episodes of severe electrolyte derangements and dehydration that are often fatal [35]. Survivors may develop chronic renal failure. Therapy has improved prognosis considerably and includes potassium supplementation, high salt intake and indomethacin (2–5 mg/kg/day); in some, spironolactone may be of benefit [35]. Often confused with the classic Bartter syndrome is the disorder first described by Gitelman [36], which consists of hypokalemic alkalosis with magnesium wasting, increased urinary excretion of prostaglandin E2, and mild elevation of plasma renin and aldosterone levels. Gitelman syndrome is hereditary (probably autosomal recessive), although most reports are of isolated patients [36]. Sodium wasting is not clinically evident under most circumstances; patients are asymptomatic except during occasional episodes of weakness, tetany, abdominal pain, vomiting, and hypokalemia. Hypocalciuria is another feature that distinguishes these patients from those with classic Bartter syndrome [36]. Therapy consists of magnesium and potassium supplementation.

Proximal tubular bicarbonate wasting is most often encountered in patients with generalized tubular dysfunction, the renal Fanconi syndrome. However, a small number of children have been described with isolated proximal renal tubular acidosis [37]. Most patients have been males without a family history of tubular dysfunction; others cases have been familial [37–39]. Nephrocalcinosis, hypokalemia, hypocitraturia, and metabolic bone disease are absent. These patients have done well on high-dose bicarbonate therapy (15–20 meq/kg/day) with resolution of acidosis and of growth failure. The pathogenetic mechanism is unknown.

Two additional hereditary syndromes of proximal renal tubular acidosis have been documented. In one Norwegian family, proximal renal tubular acidosis was associated with mental retardation, corneal opacities, glaucoma, and hypothyroidism [40]. Twelve families with proximal renal tubular acidosis, cerebral calcifications, osteopetrosis, and deficient carbonic anhydrase II activity were described recently [41]. Affected patients had absence of carbonic anhydrase II (enzymatic activity and immunoprecipitable protein was absent in red blood cell extracts) [42]. This isozyme is the principal cytoplasmic isozyme in kidney, distributed throughout the proximal tubule, distal tubule

and collecting duct. As a consequence, these patients were shown, to excrete as much as 25% of the filtered bicarbonate load, consistent with a disturbance in proximal tubular reabsorbtion [41]. A modest defect in distal hydrogen ion excretion was also present, since these patients were unable to achieve maximal urinary acidification [41]. Clinically normal parents have half-normal levels of the isozyme, as expected when the inheritance is autosomal recessive. The structural gene for carbonic anhydrase II has been mapped to chromosome 8, cloned, and shown to be polymorphic in human populations [41]. Molecular analysis of the mutation has not yet been reported, but clinical variability suggests that there may be some genetic heterogeneity. Molecular diagnosis and carrier detection should be possible.

HEREDITARY DISEASE OF DISTAL HYDROGEN ION SECRETION: DISTAL RENAL TUBULAR ACIDOSIS (DRTA)

Classical DRTA

The classical form (type I) of distal renal tubular acidosis (DRTA) is due to a defect in hydrogen ion secretion by cells of the collecting tubule. In untreated patients, there is failure to acidify the urine below pH 5.5 so that the daily acid load cannot be excreted fully. This is normally accomplished by an electrogenic proton-translocating ATPase located in the luminal membrane of the distal tubule; secreted hydrogen ions combine with ammonia and other urinary buffers to allow continued net flux of protons into the lumen. Integrity of hydrogen ion secretion is also important for complete reabsorption of the small amount of filtered bicarbonate, which is delivered distally. Thus, patients with DRTA are also found to have modest bicarbonaturia at normal plasma bicarbonate concentrations [43].

The precise physiologic defect has not yet been identified, but it does not involve aldosterone deficiency or failure of aldosterone-dependent potassium secretion in the cortical collecting duct. Because of failure of distal Na^+-H^+ exchange, sodium is lost in association with bicarbonate, and patients are typically volume-contracted; aldosterone levels are elevated and there is stimulation of potassium secretion, causing marked hypokalemia in untreated patients [44].

Another hallmark of classic DRTA is hypercalciuria with nephrocalcinosis. Chronic acidosis causes dissolution of bone and hypercalciuria [45]. In addition, acidosis stimulates mitochondrial oxidation of citrate, reducing renal excretion of this important calcium chelator [46, 47]. Together, the hypercalciuria, hypocitraturia, and high urinary pH lead to nephrocalcinosis and nephrolithiasis.

Classical DRTA presents in the first months of life with acidosis, failure to thrive, hypokalemia, dehydration, and nephrocalcinosis. Unlike adults, whose distal reclamation of bicarbonate is less than 5% of the filtered load, infants normally reabsorb 5%–15% of filtered bicarbonate in the distal nephron [43]. Thus, infants with DRTA have severe bicarbonate wasting as well as deficient

net hydrogen ion excretion and are subject to life-threatening acidosis and volume contraction. These infants were once thought to have a completely different disorder (type III RTA) from type I DRTA. Longitudinal studies indicate that the two are different stages of the same disease [48]. The designation RTA type III has been dropped [43].

Classical isolated DRTA may occur sporadically or in families as an autosomal dominant disease. Other forms of DRTA have been reported in association with a variety of hereditary systemic diseases including Ehlers-Danlos [49], elliptocytosis [50], and sickle-cell anemia [51]; the pathogenesis is uncertain. An autosomal dominant form of DRTA with nerve deafness has been described [52–54]. Shapira et al. [52] found absence of red cells' carbonic anhydrase (CAI) in one family, and proposed that this might be the primary defect giving rise to DRTA. This has not been confirmed by other investigators [55]. Moreover, Sly has pointed out that the isozyme of carbonic anhydrase (CAI) studied by Shapira is not expressed in the kidney [42]. Sly et al. identified three siblings with autosomal recessive RTA and osteopetrosis in association with absent activity of the major renal carbonic anhydrase isozyme [42]. However, some of these patients were able to acidify the urine to pH 5.4, suggesting that the mutation causes acidosis primarily through disruption of proximal tubular bicarbonate reabsorption and only to a lesser degree through blunted secretion of protons [56]. This interesting kindred is discussed above in conjunction with other forms of proximal RTA.

HEREDITARY DISEASES AFFECTING RESPONSIVENESS OF THE RENAL TUBULE TO HORMONES

Several specific reabsorptive processes in the renal tubule are highly regulated by hormones: 1) phosphate reabsorption in the proximal renal tubule is inhibited by the action of parathyroid hormone; 2) secretion of hydrogen ion and potassium in the distal nephron is augmented by aldosterone: and 3) passive transcellular reabsorption of water through vasopressin-activated membrane pores occurs in the collecting duct. Each of these hormonal interactions with the kidney is complex; hormones must bind to specific receptors, initiate a cascade of intracellular signals, and modulate the cellular machinery that supports each transport process. It is not surprising that mutations might arise that would disrupt such a long chain of events at some point. Furthermore, it is easy to see that any such process could be deranged by a variety of mutations arising at independent loci, all producing a pattern of unresponsiveness to one hormone. Nephrogenic diabetes insipidus will be discussed as the prototype for this group of hereditary tubular disorders.

Nephrogenic diabetes insipidus (NDI)

The first report of families with nephrogenic diabetes insipidus is probably that of McIlraith in 1892 [57]. During the mid-1940s, the X-linked pattern of inheritance and the complete unresponsiveness of the kidney to exogenous

vasopressin were established [58–60]. Typically, males bearing the X-linked NDI mutation come to medical attention during the first year of life because of severe failure to thrive, episodic bouts of fever, and dehydration. Polyuria is presumably manifest from an early stage of fetal life, since polyhydramnios is often noted, but this symptom and the intense polydipsia may go unnoticed during the first months of life. In the past, many affected males died in infancy or were left with significant mental retardation when untreated. It is now generally accepted that early therapy to ensure replacement of urinary free-water losses fully prevents brain damage and restores growth and development. Thus, diagnosis and medical intervention during early infancy are critical to prognosis [61–63]. Since the hypothalamic thirst mechanism remains intact, older patients are able to regulate their own serum osmolarity if allowed free access to water.

The precise defect in X-linked NDI is not known, but it is well established that the normal increase in urinary osmolarity in response to exogenous or endogenous vasopressin is absent [64]; basal urinary osmolarity is about 50–125 mosm/L and does not increase in response to 10–20 micrograms of 1-desamino (8-D-arginine) vasopressin (dDAVP) administered intranasally (65). Endogenous levels of vasopressin may be high or normal, and the ratio of endogenous vasopressin concentration to urinary osmolarity is greatly perturbed [63]. The expected increase in urinary cyclic AMP in response to dDAVP is absent in affected males [65, 66]. This suggests that the defect resides in the vasopressin receptor itself or in very early transduction events.

The vasopressor response to vasopressin is mediated by nonrenal receptors (V1) located in peripheral blood vessels. These receptors act through a phosphatidylinositol mechanism and are unresponsive to the vasopressin analogue dDAVP [64]. It is believed that the vasoconstrictor response to vasopressin is intact in NDI patients. However, there is recent evidence that renal-type receptors (V2) are also found in blood vessels and mediate the release of clotting factor VIIIc and von Willebrand factor as well as a vasodilatation [67]. Bichet et al. [64] have demonstrated that these extrarenal V2 effects are absent in NDI males following dDAVP infusion; female carriers have a blunted or partial response. This test appears to be useful in identifying female carriers.

In the initial families reported by McIlraith [57], Forssman [60], and Williams and Henry [59], NDI was transmitted as an X-linked recessive disorder. Fifty percent of males were fully affected, there was no male–male transmission, and in affected females the disease was mild or silent (about one third of obligate carriers had no evidence of clinical disease). Bode and Crawford [68] suggested that most North Americans with NDI were descended from the Ulster Scot clan, which arrived in Nova Scotia on the ship Hopewell. These kindreds conform to a pattern of X-linked inheritance. Evidence for alternate mutations causing NDI is weak. There are reports of patients with an apparent increase of urinary cyclic AMP following dDAVP administration [69], suggesting a second type of NDI. However, the accuracy of this test is debatable;

Table 8–2. Conditions characterized by tubular unresponsiveness to hormones

Disorder	Clinical findings	Pathophysiology	Genetics	References
Nephrogenic diabetes insipidus	Presentation in infancy Polyuria, polydipsia	Defective renal (V2) receptor mechanism for vasopressin in collecting duct and extrarenal sites	X-linked An autosomal subtype has been proposed but not substantiated	57–75
Pseudohypo-aldosteronism type I	Neonatal onset of hyperkalemia, hyperchloremic acidosis and urinary salt wasting Failure to thrive occasionally fatal	Renal tubular unresponsiveness to aldosterone blunts distal reabsorption of sodium and excretion of potassium and hydrogen ion	Autosomal recessive Mineralocorticoid receptor on chromosome 4 defective	87–93
	Very high serum renin, aldosterone Responds to oral salt supplementation Life-threatening electrolyte disturbances resolve by second year of life	Sweat glands, colon and salivary glands also unresponsive to aldosterone Renal Na-K-ATPase is decreased	A possible autosomal dominant form has been reported	
Pseudohypo-aldosteronism type II	Mild hyperkalemia, hyperchloremic acidosis and hypertension in adults Usually asymptomatic but occasional episodes of muscle weakness	Uncertain Possible mechanisms include 1) unresponsiveness to atrial natriuretic peptides; 2) increased chloride permeability of the distal nephron	Autosomal dominant Rare	94–99
Pseudohypo-para-thyroidism type Ia	Hypocalcemia, hyperphosphate-mia Short metacarpals/ metatarsals Dental defects Subcutaneous calcifications Mild bone demineralization Elevated serum PTH Short stature Obesity Mild mental retardation	Defective renal responses to PTH (phosphaturia, cAMP release, vitamin D synthesis) Defective responses to other cAMP-mediated hormones (thyrotropin, gonadotropin) 50% reduction in Gs protein coupling hormone receptors to adenylate cyclase	Autosomal dominant M:F ratio = 2:1 No antenatal diagnosis No deletion noted in Gs gene, but level of mRNA for Gs protein is reduced	76–82

Table 8-2 (continued)

Disorder	Clinical findings	Pathophysiology	Genetics	References
Pseudohypo-para-thyroidism type Ib	Hypocalcemia, hyperphosphatemia Normal physical appearance Normal intelligence Prominent demineralization of bone and often osteitis fibrosa cystica Elevated serum PTH	Defective response to PTH (phosphaturia, release of cAMP, and synthesis of vitamin D) Normal Gs protein activity Bone response to PTH presumed intact	Often familial but pattern of inheritance uncertain Sometimes isolated cases	83–85
Pseudohypo-para-thyroidism type II	Hypocalcemia, hyper-phosphatemia Normal physical appearance Normal intelligence Elevated serum PTH	Defective phos-phaturic response to PTH Normal urinary cAMP response	Usually sporadic One familial case (2 brothers) Possibly an acquired disease	86

in one report, which claimed a modest increase (25%) in urinary cyclic AMP following vasopressin in several Norwegian males with NDI, the authors were unable to document a significant response in control patients [69]. Cannon [70] investigated a large family in which there were several cases of male–male transmission, but some of these patients were apparently descended from the Hopewell Scots, who clearly have an X-linked mutation; consanguinity could explain the apparent male–male transmission. The number of affected males and the mild expression in female carriers in Cannon's study are typical of X-linked inheritance. NDI has been reported in aborigines from central Australia [71], a Samoan kindred [72], and two American Black kindreds [73]; we have recently investigated a Black family from Uganda with no apparent racial admixture. In these diverse racial groups, the clinical features and apparent X-linked mode of inheritance were comparable to NDI in Caucasians of European extraction. Recent studies with cDNA probes indicate that the NDI mutation maps to the Xq28 region in families from the Netherlands and in an Indiana kindred [74, 75]. Thus, the genetic evidence favors the view that NDI is caused by mutation at a single locus on the short arm of the X chromosome. The mutation may have been dispersed among the populations of the world at an early point in man's evolution, or it may represent frequent different spontaneous mutations at a susceptible locus.

The cDNA probe used by Knoers et al. (DXS52) [75] appears to be very tightly linked to the NDI locus (LOD score of 8.97), and might be an excellent clinical tool for RFLP analysis of affected families if there is adequate polymorphism in the population. Use of the probe should allow accurate carrier

detection and premorbid diagnosis. There is also the prospect that it will lead to isolation of the NDI gene and definitive answer as to whether there are subtypes of NDI.

Pseudohypoparathyroidism

Syndromes of pseudohypoparathyroidism have in common an unresponsiveness of the renal proximal tubule to parathyroid hormone (PTH). Serum levels of parathyroid hormone are elevated or high-normal. Following infusion of PTH, the phosphaturic response is blunted or absent, and basal serum levels of phosphate are elevated. In many, but not all, hypocalcemia is present, causing symptoms such as tetanic convulsions, laryngospasm, and paresthesias. Considerable heterogeneity exists with regard to additional clinical features and the underlying pathophysiology. At least three primary subtypes are recognized, as outlined in table 8-2 [76–86].

Pseudohypoaldosteronism

In the distal convoluted tubule and cortical collecting tubule, aldosterone drives the reabsorption of sodium and stimulates secretion of potassium and hydrogn ion. Two hereditary syndromes have been described in which this process is perturbed. These are designated pseudohypoaldosteronism types I and II.

In the first, pseudohypoaldosteronism (PHA) type I, infants present with severe hyperkalemia, hyperchloremic acidosis, and salt-wasting with extremely high levels of renin and aldosterone [87–89]. Infants improve dramatically when supplemented with oral sodium chloride and become asymptomatic after several years. This autosomal recessive disorder reflects complete tubular resistance to aldosterone [90]. Bosson et al. [91] described two offspring of a first-cousin marriage who presented with severe salt-wasting. Generalized pseudohypoaldosteronism was diagnosed on the basis of hyponatremia, hyperkalemia, and markedly elevated sodium concentration in urine, sweat, saliva, and stool, in the presence of increased plasma aldosterone. The parents were investigated on normal and sodium-restricted diet; the values were apparently normal on both diets. Aldosterone-binding studies performed on mononuclear leukocytes showed no type I receptors in the child, whereas low levels were found in both of the parents. Speiser et al. [92] reported two new cases of pseudohypoaldosteronism and demonstrated complete mineralocorticoid unresponsiveness in kidney, salivary glands, and sweat glands.

Arriza et al. [93] used low-stringency hybridization with the human glucocorticoid receptor cDNA to isolate a related gene encoding a high-affinity aldosterone receptor. By testing the cDNA against a panel of rodent–human somatic-cell hybrids, this mineralocorticoid receptor was mapped to human chromosome 4 [93]. It remains to be determined whether this locus is the site of the mutation in type I PHA.

The second syndrome, pseudohypoaldosteronism type II, includes patients

who come to attention later in life with mild hyperkalemia, hyperchloremic acidosis, and mild hypertension (in adults). In this latter disorder, there is minimal effect of mineralocorticoid administration on deficient potassium and hydrogen ion secretion, but distal reabsorption of sodium is excessive even under basal conditions and leads to volume expansion despite normal or low levels of aldosterone in serum [94]. Hyperkalemia resolves when treated with salt restriction and/or hydrochlorothiazide [95]. Patients with the Gordon syndrome [95, 96] and Spitzer–Weinstein syndrome [97] fall into this category and may have a common etiology. Various pathophysiologic explanations have been suggested, including hyperpermeability of the distal nephron to chloride [98]. Recently, Semmekrot et al. [99] reported renal resistance to infusion of atrial natriuretic hormone in a 14-year-old boy with the disorder. Further studies will be required to determine whether renal unresponsiveness to ANP is the primary defect.

Thus, the primary defect in type I PHA is a specific deficiency of the high-affinity receptor for mineralocorticoids. There is coexistence of salt-wasting and potassium retention, and the defect is expressed in a variety of tissues. By contrast, the primary abnormality in type II PHA is thought to be a specific defect of the renal secretory mechanism for potassium; the kaliuretic response to mineralocorticoids is limited, but sodium and chloride reabsorption is intact.

MULTISYSTEM DISEASES AFFECTING MULTIPLE REABSORPTIVE PROCESSES IN A NEPHRON SEGMENT

Several hereditary multisystem diseases affect a wide range of reabsorptive processes in a particular nephron segment. Unlike the conditions discussed above in which mutations directly alter a specific transport mechanism, these disorders presumably compromise some central aspect of cell function such as energy metabolism, leading to broad disruption of many different transport processes. When the proximal tubule is the primary target, patients develop a characteristic pattern of abnormalities originally described by De Toni, Debre, and Fanconi in the 1930s and now usually referred to as the renal Fanconi syndrome [100]. Abnormalities include hyperaminoaciduria, glucosuria with rickets, proximal renal tubular acidosis, salt-wasting and polyuria, hypokalemia, hypouricemia, and proteinuria. When untreated, the depletion of fluid and electrolytes is associated with severe failure to thrive. Proximal tubular dysfunction is the dominant clinical manifestation of cystinosis in its early phase. Cystinosis will be discussed as the prototype of these disorders. Other conditions causing the renal Fanconi syndrome are presented in table 8-3.

Cystinosis

Cystinosis is a rare autosomal recessive disorder characterized by intralysosomal accumulation of cystine in tissues throughout the body. Although the kidney and eye appear to be particularly vulnerable, elevated levels of cystine

Table 8-3. Conditions causing the renal Faconi syndrome

Condition	Clinical presentation	Basic Defect	Genetics	References
Cystinosis Infantile nephropathic form:	Failure to thrive Fanconi syndrome Rickets Acidosis Dehydration Retinopathy	Accumulation of cystine in lysosomes probably due to a defect in cystine transport at the level of the lysosomal membrane	All three forms inherited as autosomal recessive Prenatal diagnosis and heterozygote detection available	101–115
Adolescent nephropathic form:	Photophobia Retinopathy Chronic headaches Proteinuria Partial Fanconi syndrome			
Benign form:	Asymptomatic			
Cytochrome-C-oxidase deficiency	Fatal infantile mitochondrial myopathy	Cytochrome-C-oxidase activity markedly reduced in skeletal muscle and kidney	Probably autosomal recessive	119–120
	Lactic acidosis	Cytochrome-C-oxidase is an enzyme complex composed of at least 7 subunits, 3 of which are determined by mitochondrial DNA. Some of subunits encoded by nuclear genes have more than one tissue-specific isozyme	Mitochondrial gene mutations may also exist	
Fanconi–Bickel syndrome	Fanconi syndrome with fever, vomiting, rickets and growth failure. Hepatorenal glycogenosis with protuberant abdomen hepatomegaly, moon-shaped facies Delayed eruption of teeth and puberty Bone fractures and pancreatitis	Unknown	Autosomal recessive	121

Table 8-3 (continued)

Condition	Clinical presentation	Basic defect	Genetics	Reference
Fructose intolerence, hereditary	Intake of fructose or sucrose at time of weaning leads to poor feeding, vomiting, failure to thrive, hypoglycemia, shock and liver disease	Deficiency of fructose-1-phosphate aldolase B of the liver, kidney cortex, and small intestine	Autosomal recessive inheritance Heterozygotes diagnosed by enzymatic testing of the intestinal biopsies RFLP within the aldolase B gene described Both structural and controller mutations may exist, possibly more than one type of structural mutation	122–124
Galactosemia	Manifestations of central nervous system pathology Cataracts Rickets Fanconi syndrome	Galactose-1-phosphate uridyl transferase deficiency	Autosomal recessive Structural gene mapped to 9p13	125
Lowe's disease	Growth and mental retardation Hypotonia Fanconi syndrome with acidosis and rickets Chracteristic eye changes (bilateral congenital cataract and glaucoma)	Unknown	X-linked Female carrier may have lenticular opacities Gene mapped at Xq25; close linkage to RFLPs that map to Xq24–q26	126, 127
Tyosinemia type I (hepatorenal)	Liver cirrhosis with malignant transformation Porphyrialike crises with CNS involvement Fanconi syndrome (may be mild)	Fumarylacetoacetase deficiency	Autosomal recessive Prenatal diagnosis possible by amniocentesis or by chorionic sampling	128–130
Wilson's disease	Hepatolenticular degeneration with central nervous system and liver damage due to copper deposition Fanconi syndrome Low serum ceruloplasmin may be found Successful treatment by orthotopic liver transplantation	Unknown	Three forms: Atypical Neurologic Hepatic Gene on chromosome 13 distal to esterase D locus Reduced ceruloplasmin gene transcription	131–134

Table 8-3 (continued)

Condition	Clinical presentation	Basic defect	Genetics	Reference
Idiopathic Fanconi syndrome	Sporadic form: early onset of Fanconi syndrome Autosomal dominant (adult) form: early onset of lactic aciduria, proteinuria. Fanconi syndrome in second decade. Gradual onset of renal failure	Unknown	Sporadic or autosomal dominant	135–138

are readily detected in bone marrow, liver, spleen, fibroblasts, leukocytes, etc. Intestinal absorption of cystine and plasma levels of cystine are normal; urinary excretion of cystine is elevated, but only to the same degree as other amino acids [100]. Cystine stone formation does not occur as it does in cystinuria.

Three main variants of the disease have been recognized: the nephropathic or infantile form, the adolescent form with late onset of renal involvement, and the benign adult form with ocular involvement but no apparent renal dysfunction [100–102].

In the infantile form, patients appear normal at birth and during the first six months of life, although renal cystine content is elevated. By one year, affected infants usually come to medical attention because of polyuria, polydipsia, dehydration, growth retardation, and early rickets. Diagnosis can be made by identification of corneal cystine deposits with slit lamp examination and by assay of cystine in leukocytes or fibroblasts. A swan-neck deformity of the proximal tubule becomes apparent on renal biopsy in the first years of life [103]. This probably coincides with onset of the renal Fanconi syndrome, which is the dominant clinical manifestation of the disease during the first 5–10 years. Mental development is nomal; photophobia and retinopathy are usually mild at this stage. Supportive therapy is aimed at replacement of potassium, bicarbonate, phosphate, and fluid losses. Most patients require vitamin D and caloric supplementation.

During the second half of the first decade, there is progressive decrease in glomerular filtration rate. The requirement for oral supplements of potassium, phosphate, and bicarbonate diminishes gradually. By 10–11 years of age, most patients reach end-stage renal failure requiring dialytic therapy or transplantation.

Renal transplantation does not improve manifestations of cystinosis in other organs [104]. Growth failure persists, and ocular involvement may become debilitating. Hypothyroidism, insulin-dependent diabetes, encephalopathy, and

hypersplenism have been reported [105–106]. Modest hepatomegaly is common, but liver function does not deteriorate markedly. Cystine reaccumulates in the interstitium of the grafted kidney, but only as a result of infiltration by host leukocytes. Renal Fanconi syndrome does not recur and graft survival is no worse than in noncystinotic children [104]

There is now good evidence that the cystinosis mutation somehow interfers with normal efflux of cystine from the lysosome via its specific transport mechanism [107–110]. Cells that have high rates of lysosomal protein degradation generate a large intralysosomal burden of cystine that is unable to exit into the cytoplasmic compartment. Activity of cytoplasmic enzymes that could reduce and further metabolize cystine are normal. It is presumed that cystine accumulation is somehow toxic or mechanically damaging to lysosomal function, accounting for broad cellular injury. The strongest argument in favor of this pathogenetic mechanism is the observation that the reducing agent, cysteamine, given orally to 50 cystinotic children in an average dose of 50 mg/kg/day, was shown to lower the leukocyte content of cystine, improve growth, and slow progression of renal failure [111]. Late treatment does not reverse established renal damage, but early treatment may alter the course of the disease [112].

The late-onset nephropathic form (intermediate or adolescent cystinosis) starts between 18 months and 17 years of age with proteinuria due to glomerular damage, rather than with the manifestations of tubular dysfunction that mark the onset of infantile cystinosis [101]. Renal Fanconi syndrome is mild or incomplete. Additional features are chronic headaches and late development of pigmentary retinopathy. The bone marrow and cornea have cystalline deposits. Growth may be near normal or delayed [101]. The benign form (adult onset) of cystinosis presents with evidence of cystine crystal deposition in the bone marrow and the cornea, but not in the kidney [101]. Renal function is normal.

In all three forms of cystinosis, inheritance of the disease follows the autosomal recessive pattern. It is not known whether the adolescent and benign adult variants are due to the same defect in lysosomal cystine efflux as the classical infantile form, Conceivably, these diseases might be caused by mutation at other genetic loci. If the mutations are allelic, having arisen at a unique cystinosis locus, the adolescent or late-onset nephropathic cystinosis patients could be genetic compounds who have inherited one gene for the severe infantile form and one gene for the benign form. Since the cystinosis gene has not yet been mapped or cloned, it is not possible to test these various hypotheses.

Heterozygotes with infantile nephropathic cystinosis can be detected by measuring the cystine content in skin fibroblasts, amniocytes, or leukocytes. The mean free cystine level is 5–6 times greater than the normal value [113]. Smolin et al. [114] have reported an improved method for heterozygote detection by measuring cystine in isolated polymorphonuclear leukocytes rather

than in total leukocytes. Heterozygotes can also be distinguished from normal by assessing efflux of 35[S]-cystine dimethyl ester from preloaded leukocytes [108]. The mean efflux half-time is intermediate between normals and cystinotics. Gahl et al. [110] found that heterozygotes exhibit about half-normal rates of cystine counter transport into isolated leukocyte lysosomes. While these assays may be impractical for widespread clinical use, they demonstrate a gene-dosage effect that supports the conclusion that the primary defect in infantile nephrophathic cystinosis is the impaired lysosomal efflux of cystine. Prenatal diagnosis of cystinosis by estimation of the free cystine content of cultured amniotic cells is now a well-established procedure. The cystine content can also be measured in chorionic villi [115]. Premorbid diagnosis of cystinosis is important because of the evidence that early treatment with cystine-depleting agents such as cysteamine may prevent or greatly delay renal damage.

CONCLUSIONS

In this chapter, we have reviewed the hereditary tubular transport disorders. Although the number and diversity of these conditions is somewhat daunting, we have grouped them into five basic patterns of tubular pathophysiology. In most (e.g., cystinuria), the mutant gene product is unknown, and therefore diagnosis depends on accurate identification of a specific urinary phenotype. Furthermore, most of these disorders involve transport mechanisms that are expressed only in kidney and other absorptive epithelia so that the mutation cannot be identified in fibroblasts or amniocytes. Consequently, it is with great expectations that we look ahead to the next decade of molecular biology. Already, the genetic loci for certain of these tubular disorders have been mapped roughly. Identification of flanking gene markers will allow carrier detection and antenatal diagnosis in families known to bear the mutation. In conditions such as nephrogenic diabetes insipidus, early diagnosis will allow rapid medical intervention. Finally, we are certain to see the isolation and characterization of individual genes specifically involved in many of these disorders. Sequence analysis of the mutant genes should unravel many of the questions about heterogeneity in each disorder; analysis of the corresponding normal genes should identify some of the elusive transport proteins and related peptides that govern tubular physiology in man.

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9. GENETICS OF VITAMIN-D-RESISTANT RICKETS

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Vitamin-D-resistant rickets (VDRR) is a term used to describe several different genetic disorders of phosphate and vitamin D metabolism that are refractory to the treatment regimen used for vitamin-D-deficiency rickets. Thirty years ago it was felt that all cases of VDRR were due to a single X-linked genetic disorder [1, 2]. Because hypophosphatemia was a prominent feature, the disease was called either X-linked hypophosphatemia (XLH) or familial hypophosphatemia (McKusick catalogue, MIM 30780). While XLH continues to account for the majority of VDRR cases, since 1960 there have been numerous reports of non-X-linked VDRR, including both autosomal dominant [3] and autosomal recessive [4, 5] forms. In addition, two human X-linked genes that may be implicated in VDRR have been described [6]. These findings indicate that the genetic regulation of renal phosphate transport is more complicated than was thought previously [7, 8]. The purpose of this chapter is to review the mode of inheritance of various forms of VDRR. The emphasis will be placed on XLH, which is the best-characterized condition of this group.

BACKGROUND

At the beginning of the twentieth century, in the vast majority of cases, rickets was secondary to vitamin D deficiency. The introduction of vitamin D has virtually eliminated the deficiency state as a cause of rickets [9, 10], and VDRR became noticeable. In 1938, Albright et al. [11] described a boy who required

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massive doses of vitamin D to heal his rickets. Several years later, Christensen [12] postulated that autosomal dominant inheritance was responsible for a mother, son, and daughter with rickets unresponsive to standard vitamin D therapy. In 1957, Winters et al. [1] were able to identify an X-linked pattern of inheritance of a vitamin-D-resistant form of rickets in a large North Carolina kindred by using hypophosphatemia to separate affected subjects and carriers from noncarriers. Subsequent reports by the same [2, 13] and other [14] investigators confirmed the conclusion that vitamin-D-resistant hypophosphatemic rickets has an X-linked mode of inheritance. Sporadic cases were also identified [14, 15].

The affected individuals had normal parents as determined by history, physical examination, radiological evaluation, and blood chemistry analyses. Burnett et al. [14] described eight patients, both males and females, with clinical features similar to those of XLH in whom the disease appeared to be the result of new mutations. The single subject reported by Winters et al. [15] was that of a female who was more severely affected than typical XLH males. The authors speculated that a different gene, perhaps autosomal recessive, or an autosomal dominant new mutation might be responsible for the disease. Subsequently, both autosomal dominant and recessive forms of VDRR have been confirmed to exist.

X-LINKED VITAMIN-D-RESISTANT RICKETS

General characteristics

XLH is a disorder characterized by hypophosphatemia, secondary to a reduced capacity of the renal tubules to reabsorb phosphate. This leads to hyperphosphaturia, hypophosphatemia, rickets, and growth retardation. The serum concentration of calcium is normal, that of 1,25-dihydroxyvitamin D_3 low-normal to normal (untreated state), the parathyroid hormone level is usually normal, the alkaline phosphatase is high, and the urinary calcium concentration is low [16, 17]. A more detailed description of the clinical, radiological, and biochemical characteristics can be found elsewhere [16, 17]. Hemizygous males are more likely to have more severe skeletal changes and growth retardation than are heterozygous females. However, the expression of the disorder is variable, and heterozygous females can be affected as severely as males [16]. Variation in the degree of XLH:normal X-chromosome inactivation (Lyon hypothesis) is assumed to account for this variability in expression [18]. The presence or absence of skeletal abnormalities is an inadequate test of carrier status. The preferred phenotypic marker for the gene defect is hypophosphatemia; yet absence of hypophosphatemia does not exclude the carrier status.

Hyp mouse investigations

For the 30 years that followed the initial description of the X-linked inheritance pattern of XLH, studies were directed toward the elucidation of the specific defect in phosphate metabolism. Discovery of the hypophosphatemic

(Hyp) mouse [19] has provided a model for the human disorder [8, 16]. Like that of the human, the disease of the Hyp mouse is X-linked and characterized by hyperphosphaturia, hypophosphatemia, rachitic bone changes, and growth retardation; the gene defect is considered to be analogous to that of human XLH [19, 20].

Experiments performed in brush border membrane vesicles prepared from kidneys of Hyp mice have revealed a low Na^{+}–Pi uptake [21]. There is also a deficiency in renal 1α-hydroxylase, the enzyme that converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D [22]. The fact that serum 1,25-dihydroxyvitamin D_3 concentration is low, in spite of hypophosphatemia [23], indicates that a similar defect exists in humans. It is, however, unclear how the defect in phosphate transport may be related to the hydroxylation of 25-hydroxyvitamin D. The possibility has been considered that an abnormality in cyclic AMP-dependent protein kinase or in protein kinase inhibitor activity may account for both the defect in phosphate transport and the defect in the synthesis of 1,25-dihydroxyvitamin D_3. In the Hyp mouse, however, there is a significantly elevated renal protein kinase C activity that is not found in other tissues [24]. The significance of this finding for human disease remains unknown.

Investigation of XLH by molecular biologic techniques

The specific location of the XLH gene on the X-chromosome was unknown until 1986. Based on the Hyp locus in the mouse, it was predicted that the gene would be located either on the distal short arm or the middle segment of the long arm of the human X-chromosome [25]. Using restriction fragment length polymorphism (RFLP) analysis, Read et al. [27] and Mächler et al. [28] found the DNA markers 99.6 (DXS41) and D2 (DXS43) to be linked to the XLH locus. These DNA markers are located on the short arm of the X-chromosome in the region Xp22.1–Xp22.2 [29]. Read et al. [27] studied eleven families with XLH. For marker 99.6, the LOD score was 4.82 at 0.10 recombination (a LOD score of ≥ 3.0 is considered proof of linkage [26]), and for marker D2, the LOD score was 1.96 at 0.15 recombination. Machler et al. [28] investigated two families with XLH and found a LOD score of 5.084 at zero percent recombination for marker 99.6, and a LOD score of 2.527 at 0.06 recombination for marker D2. Combining the reported LOD scores at recombination intervals of 0.05 gives a maximum LOD score for marker 99.6 of 8.94 at 0.10 recombination and for marker D2 of 4.36 at 0.10 recombination. Recently, Thakker et al. [30] analyzed several more affected families. Using multipoint mapping analysis of four DNA markers (99.6, D2, 782, 754) and the XLH locus, these investigators have constructed a linkage map of the region surrounding the XLH locus (figure 9-1). According to this map, the XLH locus is flanked by the DNA markers 99.6 and D2 at recombination fractions of 0.11 and 0.14, respectively. This means that the 99.6 locus recombines with the XLH locus at a rate of 11 times per 100 meiotic events

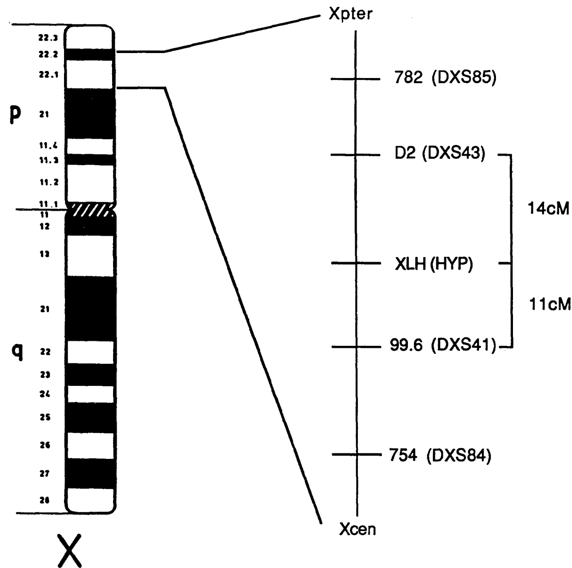

Figure 9-1. Linkage map of DNA markers surrounding the XLH region at Xp22. DNA markers are shown at relative positions along the Xp region, with both marker name and Human Gene Mapping Workshop number (DXS . . .). The XLH locus is designated by the HGM Workshop gene name HYP (formerly HPDR). For the markers 99.6 and D2, the genetic distance is shown in centimorgans (cM; 1 cM equals 1% recombination). Adapted from data in Thakker et al. [30].

(parental formation of gametes) analyzed; the D2 locus recombines at a rate of 14 times per 100 meioses.

An example of DNA analysis of a family with an affected father (II-2), unaffected male (II-1) and female (II-4), and an affected daughter (III-1) is shown in figure 9-2. The mother (I-1) of the affected male was not a carrier for XLH by standard criteria, and there was no significant family history. Southern blot analysis was performed using the DNA marker 99.6 and the restriction enzyme Pst I. The males demonstrated a single upper or lower allele for this X-linked DNA marker, whereas the females expressed two copies of either the upper or the lower allele (homozygous for RFLP) or a combination of one upper and one lower allele (heterozygous for RFLP). Therefore, in this family, XLH has occurred as a result of a new mutation. Since the affected male is hemizygous for the lower RFLP allele, it would be expected that his daughter has inherited XLH in association with his lower allele.

Further steps toward understanding the gene defect in XLH include the development of more closely linked markers and progressive isolation of overlapping DNA segments between these markers (chromosome walking or hopping), with the ultimate goal of isolating the gene and its protein product. This *reverse genetics* approach, that starts with the isolation of the gene and

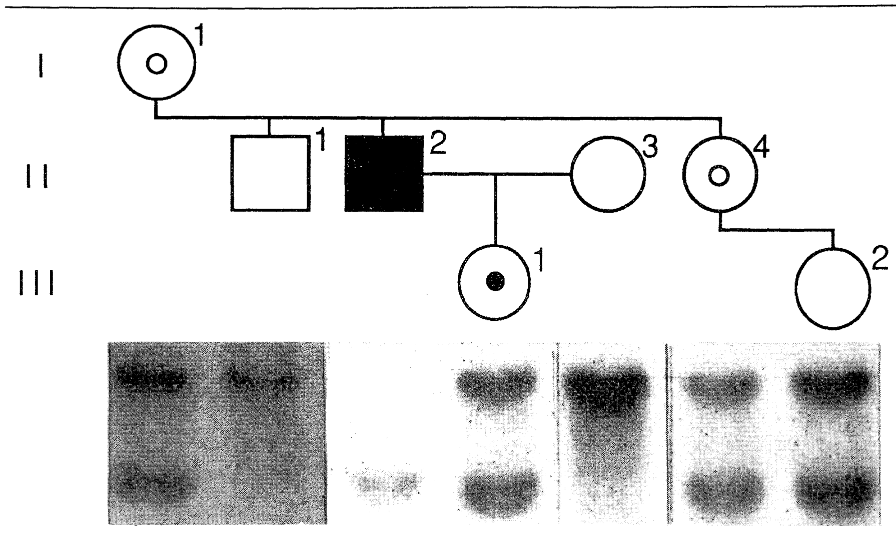

Figure 9-2. Southern blot analysis of an XLH family (MCV #6068916) using the 99.6 DNA marker and the Pst I restriction enzyme. Pedigree symbols at top correspond to lanes of autoradiogram below. Pedigree symbols are as follows: circles with open small circle, noncarrier female by biochemical testing; circle with filled small circle, carrier female; circle with no central circle, unaffected female; open square, unaffected male; filled square, affected male.

proceeds towards the identification of the protein, was applied successfully to the investigation of the gene for Duchenne muscular dystrophy (DMD), which, like the gene for XLH, is located on the short arm of the X-chromosome [31].

Genetic heterogeneity in XLH

The Hyp mouse has been the model for the investigaion of the human XLH disorder for over 10 years. Recently, a second X-linked mouse model has been described, the gyro (Gy) mouse [32]. The Gy mouse possesses most of the features of the Hyp mouse as well as an unusual circling behavior, related to developmental abnormalities of the cochlea and vestibular apparatus. The Hyp and Gy loci are very tightly linked, and distinctly separate on the mouse X-chromosome, with a frequency of recombination of 0.004 to 0.008 [32]. The origins of these mutations are somewhat different. The Hyp mouse is the result of a spontaneous mutation during a linkage experiment [19], while the Gy mouse is the result of radiation-induced mutation [32]. These observations raised the possibility that XLH might be due to transformations at more than one locus [7, 8] and stimulated a search for two forms of the human disorder. Hearing loss in XLH patients due to cochlear dysfunction and possibly endolymphatic hydrops of the cochlea [33, 34] could represent variations in phenotypic expression that might result from XLH–Gy-type gene defects. Boneh et al. [6] performed auditory testing in several families with XLH. Five

individuals, including two mother/son pairs, were found to be concordant for auditory changes and XLH. Further studies in large kindreds are required to confirm this report. There have been no differences between XLH families in blood chemistries, radiological findings, or physical characteristics that would suggest genetic heterogeneity.

Genetic counseling in XLH

The objectives of genetic counseling are to provide information to an affected family on X-linked inheritance patterns and to evaluate the risk for carrier status in relatives and future offspring. The diagnosis must be consistent with well-defined criteria [16, 17]. Construction of a reliable pedigree requires specific questions to identify those individuals with a history of rickets, osteomalacia, or short stature. Physical examination, biochemical and radiological studies, and pedigree analysis must be performed to confirm the X-linked inheritance and to identify the carriers. Ideally, all individuals at risk for carrier status and especially those with positive historical features (rickets, short stature, etc.) should be examined and tested for hypophosphatemia. In the absence of a clear X-linked pattern of inheritance, autosomal dominant or recessive inheritance needs to be considered.

The family whose pedigree is presented in figure 9-2 is an example of the questions that arise when a definite conclusion of X-linked inheritance is not possible. The diagnosis of XLH in the affected male (II-2) and his affected daughter (III-1) is based on standard clinical, radiological, and biochemical findings. The mother (I-1) of the affected male is not a carrier for XLH, as defined by standard biochemical testing. Therefore, when one counsels such a family, autosomal dominant inheritance (new mutation in II-2) must be considered, in addition to XLH. As in all X-linked conditions, affected (hemizygous) males will always transmit the XLH gene to female offspring, and heterozygous females have a 50% risk of transmitting the XLH gene to each offspring. Although all males who inherit the XLH gene will be affected, the empiric risk for growth retardation and skeletal changes for female off-spring is less than 50%. One cannot use the experience of previous heterozygous female siblings or relatives to predict the risk of future heterozygotes because the expression of the gene, even in sibships, is variable [16].

Prenatal diagnosis is not yet available in this disorder. With DNA markers at a genetic distance of greater than 10% recombination from the XLH locus, there is an unacceptably high chance for error. Because the DNA markers themselves are 0.25 recombination units apart, there is a 25% chance of crossover between 99.6 and D2 in any single meiosis. The use of linked DNA markers in prenatal diagnosis requires the analysis of the original affected individual as well as analysis of the fetus. Therefore, there are two chances for meiotic cross-overs in the formation of gametes from the carrier parent (2 × 25%), or a total of 50% chance of a cross-over between the two markers. This is an unacceptable level of inaccuracy. In addition, many XLH families may

not see this condition as being as serious as other genetic disorders, such as chromosome aneuploidy, mental retardation syndromes, or certain inborn errors of metabolism, and may not accept the termination of pregnancy. All families, however, should be interested in prenatal or neonatal diagnosis for the purpose of early, presymptomatic institution of treatment. Ongoing research into this disorder, with more closely linked DNA markers, should ultimately lead to the identification of the XLH gene and its protein expression product. This should make it possible to establish definitive prenatal diagnostic tests and, eventually to correct the genetic abnormality.

Future prospects for XLH genetic research

The usefulness of the RFLP approach using linked probes to find a particular gene has been demonstrated for disorders such as Huntington's disease [35] and cystic fibrosis [36]. There may be some limitations, however, in the application of this method to the investigation of an X-linked gene. It has been shown that the nucleotide variation on the X-chromosome is limited, due to the conservation of a haploid state in males [37, 38]. With fewer nucleotide variations, there are fewer and less informative RFLPs detectable by DNA markers. With fewer RFLPs, isolation of the gene by the polymorphic DNA marker approach is more difficult, since one has to search harder to detect any linked DNA marker. Fortunately, new techniques in recombinant DNA technology are developing at a rapid pace, and if one approach fails, others may be utilized or adapted to the investigation of this disorder. The isolation of the DMD gene was aided immensely by the discovery of a DMD patient with chronic granulomatous disease and retinitis pigmentosa who had a deletion involving the region of interest [39]. Chromosome Xp22 deletions have not yet been described for the XLH patients, but one might expect that individuals with XLH, like those with other phenotypic, dysmorphic, or developmental disorders, are likely to demonstrate a chromosomal deletion or rearrangement. Such a rearrangement involving the XLH gene would facilitate cloning of segments from within the gene itself and would dramatically advance research in this area.

AUTOSOMAL DOMINANT AND RECESSIVE FORMS OF VDRR

There have been several reports of autosomal dominant and recessive forms of VDRR. Because the autosomal forms of VDRR are much rarer than the XLH type, only a small cohort of patients is available for study. As a result, it is not quite clear how many forms of autosomal VDRR exist. This is not unusual with regard to genetic syndromes, since questions about heterogeneity of a disorder versus a broad spectrum for a disorder (*splitting* versus *lumping*) arise often. Some distinct varieties of autosomal VDRR deserve mention. Addi-

tionally, secondary forms of VDRR have been described in association with various tumors or malignancies [40] and in conjunction with hypomagnesemia [41].

Scriver et al. [3] have described a dominant disorder that they named hypophosphatemic nonrachitic bone disease (HBD) (MIM 14635), which is characterized by impairment in statural growth, bowing of the lower extremities, and absence of rickets. These patients have hypophosphatemia, but serum values for calcium, parathyroid hormone, and vitamin D are normal. Four of the five patients reported responded to 1,25-dihydroxyvitamin D_3 therapy. A second autosomal dominant form of VDRR that had been described (MIM 19310) is characterized by hypophosphatemia with growth retardation, rickets (childhood), and osteomalacia (adulthood) with severe pain in the large joints and neck [42]. Father-to-son transmission has been observed, and 1,25-dihydroxyvitamin D_3 therapy appeared ineffective [43].

Stamp and Baker [4] have described a recessive form of VDRR characterized by hypophosphatemia, severe rickets, and unresponsiveness to 1,25-dihydroxyvitamin D_3 therapy. Other characteristics of this disorder are early fusion of cranial sutures, an increase in bone density, and nerve deafness. Two of three children were affected. The parents, who were first cousins, were both normal. A second recessive form has been described by Tieder et al. [5, 44]. Among 59 closely related individuals of a Bedouin tribe, several members had hypophosphatemic rickets with hypercalciuria (HHRH) (MIM 24153). Additional features of this disorder are short stature, hypophosphatemia, and hyperphosphaturia; serum calcium concentration is normal, but 1,25-dihydroxyvitamin D_3 concentration is elevated [26]. Phosphate supplementation reversed all clinical and biochemical abnormalities. A number of individuals in this kinship were noted to have isolated hypercalciuria and mild biochemical abnormalities. Thus, unlike in XLH, where there is a paradoxically low 1,25-dihydroxyvitamin D_3 concentration [23], in HHRH hypophosphatemia results in an appropriately high vitamin D concentration. However, the two disorders share the characteristic abnormality in tubular reabsorption of phosphate.

CONCLUSIONS

It is likely that hypophosphatemia is the common expression of diverse genetic disorders. Some of these conditions are X-linked [45], others are autosomal dominant, and others have a recessive mode of inheritance. In addition, some of the autosomal conditions may be allelic. Consequently, each affected individual should be evaluated thoroughly in order to establish a specific diagnosis [7]. This should lead to correct genetic counseling and appropriate therapeutic intervention.

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10. GENETICS OF RENAL CYSTIC DISEASES

VICENTE E. TORRES

The term *cystic diseases of the kidney* includes a large number of sporadic and genetically determined congenital, developmental, or acquired conditions that have in common the presence of renal cysts. A cyst can be defined as a gross cavity lined by epithelium or abnormal tissue and usually containing fluid or other material [1]. In the kidney, the cysts are usually of tubular origin. Beyond the definition of renal cyst, there is considerable confusion regarding the precise meaning of various terms used to describe these conditions [2]. In addition, many of the renal cystic diseases of genetic origin result from a defect in renal morphogenesis, another area characterized by imprecise terminology [3–5].

CONCEPTS AND TERMS

In order to avoid further confusion, we will follow the recommendations of the international working groups on nomenclature [2, 3]. As indicated in figure 10-1, morphologic anomalies can be due to an intrinsic (malformation) or extrinsic (disruption) defect in an embryonic developmental field, to the abnormal organization of cells into tissue (dysplasia), or to mechanical forces (deformation). A developmental field is a region of an embryo that interacts as a unit and generates a complex anatomical structure. Components of a field can be contiguous (monotopic fields), or located at a distance from each other (polytopic fields). A defect in the development of a polytopic field may result in a puzzling pattern of multiple abnormalities, the common pathogenesis of

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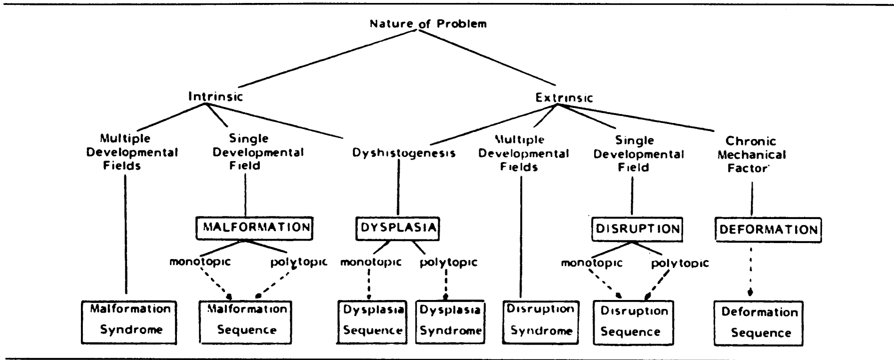

Figure 10-1. Classification of the errors of morphogenesis according to Spranger et al. [3].

which may be difficult to recognize. The terms *sequence*, *syndrome*, and *association* are used to describe multiple morphologic defects other than polytopic field defects. A sequence is a pattern of multiple anomalies that results from a single known or presumed prior anomaly or mechanical factor. A syndrome is a pattern of multiple anomalies thought to be pathogenetically related and not known to represent a polytopic field defect or a single sequence. An association is a nonrandom occurrence of multiple anomalies in two or more individuals that does not fall in any of the previous categories. Additional terms of relevance to this chapter are agenesis (absent primordium), aplasia (failure of the primordium to develop), hypoplasia (underdevelopment of an organ due to a decrease of number of cells—in the case of the kidney, the numbe of nephrons), and hypodysplasia (combination of hypoplasia and dysplasia). Since distinction between renal agenesis and aplasia is often impossible, only the term *agenesis* will be used in this chapter.

SUMMARY OF RENAL EMBRYOLOGY

To understand the pathogenesis and clinical presentation of the renal cystic diseases, it is essential to have an adequate appreciation of general morphogenesis and renal embryology. Like any other organ, the kidney interacts during embryogenesis with contiguous structures that belong to various developmental fields. The nature of the inductive interaction between different primordia at the molecular level and the translation of these molecular events into development of a form are just beginning to be understood.

Shortly after fertilization, a rapid succession of cell divisions result in the formation of the blastoderm, a flat disc on the egg membrane above the yolk, made of three germ layers (figure 10-2): the ectoderm, which ultimately develops to form the nervous system and the skin; the mesoderm, which develops into the muscles, bones, and kidneys; and the endoderm, which gives rise to the lungs, the intestines, and other organs. At the end of the third week

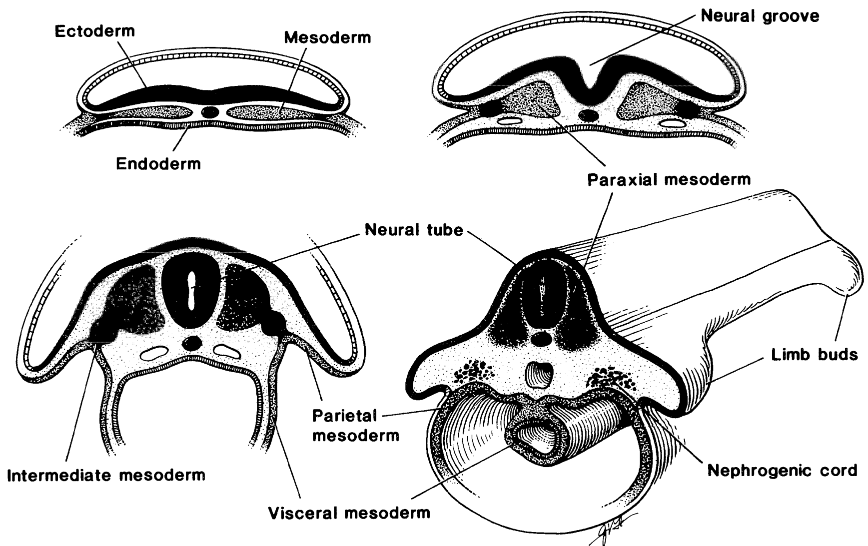

Figure 10-2. Stages of embryonic development during the third and fourth weeks.

of intrauterine life, the neural groove forms in the midline of the ectodermal cell layer. Closure of the neural groove to form the neural tube begins in the cervical region, proceeds in cephalic and caudal directions, and is complete by the end of the fourth week. By the end of the third week, the mesodermal layer has differentiated into the paraxial mesoderm, which divides into somites; the intermediate mesoderm, which forms the nephrogenic cord; and the parietal and visceral mesoderms. At the beginning of the fourth week, the cells of the somite differentiate into the dermatome, myotome, and sclerotome. During the fourth week, the cells of the sclerotome (mesenchyma) migrate around the neural tube and notochord to form the vertebral column. The branchial arches, which contribute to the formation of the neck and head, also form in the fourth and fifth weeks of development and consist of bars of mesenchyma separated by narrowed areas of tissue, located between the endodermal pharynegeal pouches on the inside and ectodermal branchial clefts on the outside. The limb buds, which consist of cores of mesenchyma and a layer of ectoderm, develop at the end of the fifth week. The limb buds and the nephrogenic cord are in very close proximity. As a result of the rapid cephalocaudal and lateral growth and folding of the embryo, a portion of the endoderm-lined cavity is incorporated into the body of the embryo to form the gastrointestinal tract. The caudal portion of this tract is the cloaca, which contributes to the development of the urinary bladder [6].

The nephrogenic cord that contains the first primitive glomeruli and tubules

(pronephros) develops in the cervical region from the intermediate mesoderm at the beginning of the fourth week [7]. The unit undergoes continuous restructuring while migrating in a caudal direction. By the end of the fourth week it has completely disappeared at the level of the cervical region. The pronephros contributes to the formation of the mesonephric or Wolffian duct. The mesonephros forms a large oval organ that disappears by the end of the second month. The formation of the metanephros or permanent kidney begins in the fifth week. The ureteral bud arises from the caudal end of the mesonephric duct and penetrates the caudal end of the nephrogenic chord or metanephric blastema. The active growing tip of the ureteral bud or ampulla undergoes repeated dichotomous branching. After the initial three to five divisions, the ampulla begins to induce the cells of the metanephric blastema to organize into S-shaped bodies, which later will differentiate into glomeruli, proximal tubules, loops of Henle, and distal tubules. The limb of the S-shaped body that develops into the distal tubule attaches to a division of the ureteral bud that will become a collecting tubule. As the ureteral bud divides and advances towards the surface, it carries the newly formed nephrons, thus explaining the absence of glomeruli in the renal medulla. The renal pelvis and calices derive from the first six divisions of the bud, while the papillary collecting ducts originate from divisions 6–10. The renal pelvis forms by 10–12 weeks, when the first divisions of the ureteral bud dilate and expand as a result of the pressure exerted by the urine excreted by the first few generations of nephrons. The chronology of the ureteral bud branching and metanephric induction was established by the detailed microdissection studies of Osathanondh and Potter and is summarized in figure 10–3 [8].

This simplified review of morphogenesis and embryology may help to understand some of the puzzling patterns of multiple abnormalities often found in association with renal cystic diseases. For example, the coexistence of renal and cerebellar anomalies in several syndromes may be due to the defective production of a common cell-adhesion molecule (CAM) or the inability of cell surface receptors to bind these molecules [9]. The simultaneous involvement of the inner ear, the branchial arches, and kidneys in the branchio-oto-renal syndrome suggests that differentiation of these organs is under the control of the same inducing or organizing mechanisms [10]. The fact that the spine and kidney develop at approximately the same time, during the fourth week, has been thought to explain the frequent association of congenital renal anomalies and neural groove defects [11]. The close proximity between the pronephros and the developing cervical spine probably accounts for the frequent occurrence of renal anomalies in the Klippel–Feil syndrome [12]. Finally, in the acrorenal field defects, the limb anomalies are thought to be due to the defective production of a limb inductor by the mesonephros. At an early stage of development, the limb buds and nephrogenic cord are in very close proximity, and it is known that mesonephric tissue is required for limb bud cartilage to continue developmet in vitro [13].

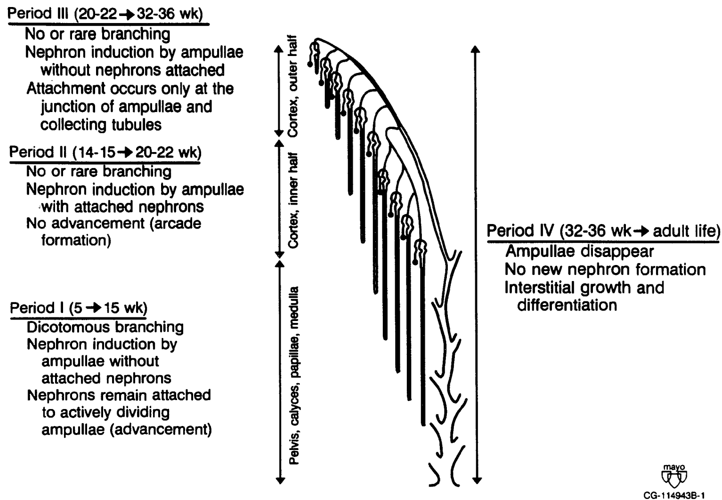

Figure 10-3. Chronology of ureteral bud branching according to Osathanondh and Potter [8].

CLASSIFICATION OF RENAL CYSTIC DISEASES

There have been many classifications of the renal cystic diseases based on morphological and clinical observations [14–16]. Unfortunately, cystic kidneys with many different etiologies may look alike, while the same etiologic entity may result in a wide spectrum of renal abnormalities. Other classifications have been based on the genetic pattern of inheritance. These classifications reduce to a simple list a very large and heterogenous group of conditions and assume that diseases for which a pattern of inheritance has not been proven are not genetic in origin [2]. Bernstein combined morphological, clinical, and genetic information in his clinical–pathologic classification and indicated that all classifications of renal cystic diseases should remain tentative to incorporate newly developed information [17]. Possibly, the ultimate classification of renal cystic diseases will be based on etiologic considerations, but at the present very little is known on the causes of cyst formation at a molecular level. The classification used in this chapter is based on previous classifications and incorporates old and new concepts regarding the pathogenesis of renal cystic disease (table 10-1). Some overlapping between the first, second, and third categories in table 10-1 can be expected, since it is likely that metabolic or structural defects present during embryogenesis may persist into later life and cause cystic transformation of well-developed nephrons—and vice versa, defects mainly expressed during adult life may manifest early and interfere with normal metanephric differentiation.

Table 10-1. Classification of the cystic diseases of the kidney

- I. Defects of metanephric differentiation (agenesis, Potter IIA or multicystic, Potter IIB or hypodysplastic, Potter IV or renal dysplasia with glomerular cysts, related renal abnormalities)
  - A. Malformations
    - 1. Single gene mutations
      - a. Dominantly inherited renal adysplasia
      - b. Branchio–oto–renal dysplasia
      - c. Cryptophthalmos-syndactyly syndrome
      - d. Acrorenal field defect
      - e. Skeletal dysplasias
      - f. Other autosomal recessive metabolic dysplasias and mutiple malformation syndromes
    - 2. Chromosomal aberrations
      - a. Numerical (e.g., trisomies 21, 18, 13)
      - b. Structural (e.g., 4p deletion)
    - 3. Multifactorial
      - a. Neural tube defects
    - 4. Sporadic syndromes or associations
      - a. De Lange syndrome
      - b. Klippel–Feil syndrome
      - c. VATER association
  - B. Disruptions
    - 1. Radiation
    - 2. Teratogenic chemicals (thalidomide, alcohol, trimethadione)
    - 3. Metabolic (diabetes mellitus, hypercalcemia, hypoxia)
    - 4. Infections (rubella)
    - 5. Teratomas (presacral teratoma)
- II. Renal cystic diseases with hereditary interstitial nephritis (nephronophthisis–medullary cystic disease complex)
  - A. Juvenile autosomal recessive
  - B. Adult autosomal dominant
  - C. Autosomal recessive forms with extrarenal abnormalities
    - 1. Senior's syndrome
    - 2. Skeletal dysplasias
    - 3. Oculo-cerebro-hepatorenal dysplasia
    - 4. Laurence–Moon–Bardet–Biedl syndrome
    - 5. Alström's syndrome
- III. Renal cystic diseases with normal metanephric differentiation and hyperplasia of the tubular epithelium
  - A. Autosomal recessive polycystic kidney disease (ARPKD)
  - B. Medullary sponge kidney (MSK)
  - C. Autosomal dominant polycystic kidney disease (ADPKD)
  - D. Tuberous sclerosis
  - E. Von Hippel–Lindau's disease
  - F. Neurofibromatosis
  - G. Oro-facio-digital syndrome type I
  - H. Glomerulocystic kidney disease
  - I. Multiple simple cysts
  - J. Acquired cystic disease
- IV. Neoplastic renal cysts
  - A. Of metanephric origin
    - 1. Multilocular cyst
    - 2. Cystic congenital mesoblastic nephroma
    - 3. Cystic nephroblastoma (Wilms' tumor)

Table 10-1 (continued)

B. Of epithelial origin
1. Unilocular or multilocular cystic renal-cell carcinoma
2. Cystic oncocytoma
C. Metastatic (adenocarcinoma, melanoma)
V. Inflammatory
VI. Cysts of nontubular origin
A. Cysts of the renal sinus
1. Hilus cysts
2. Parapelvic lymphangiectasia
B. Pelvicalyceal cysts

CYSTIC DISEASE OF THE KIDNEY DUE TO ABNORMAL METANEPHRIC DIFFERENTIATION

Osathanondh and Potter divided the renal cystic disorders into four types [14]. Type II and type IV are characterized by an abnormal branching of the tubules, which these authors interpreted to be the result of an interference with a normal ampullary activity. Some cystic kidneys were considered intermediate between type II and type IV, suggesting similar etiologic factors. In type II, the inhibition of the ampullary activity occurs very early; very few collecting ducts are formed, and very few nephrons develop. The kidney becomes a cluster of cysts with little or no residual parenchyma. Histologic features of renal dysplasia, including the presence of primitive ducts, tubules and glomeruli, and nests of metaplastic cartilage, are often present. These kidneys may be of normal or larger than normal size (multicystic or type IIA kidneys) or markedly reduced in size (hypodysplastic or type IIB kidneys). These probably represent different stages of the same pathologic process, as indicated by the demonstration that renal cysts can involute and even disappear completely during intrauterine life [18]. In type IV, the ampullary injury, which results from urethral or ureteral obstruction, mainfests at a late stage of development. Usually the obstruction is only patrial. There may be a mild irregularity in branching with a mild generalized dilatation of the collecting tubules in the medulla, but most nephrons, except the last to be formed, are normal. The cysts are found under the capsule and generally derive from Bowman's spaces, loops of Henle, or terminal ends of collecting tubules. A variety of renal abnormalities in the contralateral kidney can be found in association with unilateral type II or type IV cystic kidneys. These include renal agenesis, ectopy or fusion, and ureteral duplication or obstruction that may result from injury to the ureteral bud during various stages of development. When the injury to the ureteral bud occurs before a communication with the metanephric blastema has been established, secondary atrophy of the metanephric blastema and renal agenesis ensue. On the other hand, if the injury of the bud or ureteral obstruction occurs after renal development is

completed, dysplasia does not occur [16]. Thus, a spectrum of renal abnormalities ranging from agenesis and severe dysplasia to mild cystic dysplasia with glomerular cysts and a variety of related renal and ureteral abnormalities may result from interferences with normal ampullary activity and metanephric differentiation.

Abnormal metanephric differentiation may be the consequence of an intrinsic (malformation) or extrinsic (disruption) defect in organogenesis. An intrinsic defect may be due to a single gene mutation, a chromosomal aberration, or a combination of genetic and environmental factors (multifactorial determination). Extrinsic causes include, among others, teratogenic chemicals, metabolic abnormalities, and infections (table 10-1). Evidence for intrinsic or extrinsic defects should be sought by careful review of the pregnancy, family history, and physical examination (pattern of associated abnormalities), as well as by the study of the karyotype. Renal agenesis, hypodysplastic, and multicystic kidneys frequently occur as sporadic events.

The clinical presentation of the disorders of metanephric differentiation depends on the severity of the renal disease and associated abnormalities. A lack of fetal urine is associated with oligohydramnios, a typical facies, wrinkled skin, compression deformities of the limbs, and respiratory distress caused by pulmonary hypoplasia [19, 20]. Unilateral agenesis and hypodysplastic kidneys may go unnoticed if the contralateral kidney is normal, or they may be diagnosed incidentally later in life. Multicystic dysplasia is the most common or second most common cause of an abdominal mass in the newborn [21–24]. Sometimes it is not detected until adult age, when it may be discovered incidentally or during evaluation for abdominal or flank discomfort due to the mass effect of the lesion [25]. Differentiation of multicystic renal dysplasia from hydronephrosis in the newborn is essential because the therapeutic approaches to these conditions differ. Ultrasonography has become an important diagnostic technique in the evaluation of these patients. Excretory urography is frequently unsatisfactory in the newborn infant due to low glomerular filtration and concentrating ability]26]. Concentration of radioisotope on delayed images of DMSA or DTPA scintigraphy can be seen only in rare cases [27]. The most useful ultrasonographic criteria for identifying a multicystic kidney include the presence of interphases between cysts, nonmedial location of the larger cysts, and absence of identifiable renal sinus, all accurate in 100% of the cases,and the absence of parenchymal tissue, accurate in 73% of the cases. The diagnosis can be confirmed by retrograde pyelography showing an absent or atretic proximal ureter and by angiography revealing an absent or hypoplastic renal artery [21].

There is no agreement on the management of the asymptomatic multicystic kidney. Some investigators recommend a medical approach because multicystic kidneys tend to decreases in size with time [28]. Others recommend surgery because of the risk of hypertension and malignant degeneration [29]. Due to the fact that in most cases unilateral multicystic kidneys have been removed,

the natural history of this condition remains unclear. It should be noted, however, that there are only three well-documented cases of associated hypertension [30, 31]. There are two reports of Wilms' tumors and four of renal-cell carcinomas in patients with unilateral multicystic kidneys [31–35]. The fact that the carcinomas developed in relatively young women may support the contention that dysplastic kidneys have a propensity towards malignant degeneration.

The widespread use of fetal sonography led to an increase in the prenatal diagnosis of urinary tract abnormalities [36–39]. By this method, normal fetal kidneys can be identified by the 15th gestational week, and the bladder can be seen early in the second trimester. The most commnly detected abnormalities are obstruction, multicystic dysplasia, and renal agenesis. Coronal scans are helpful for the differential diagnosis between obstruction and multicystic dysplasia. Despite the fact that multicystic dysplasia is caused by an early interference with ampullary activity, the diagnosis is usually made during the third trimester of pregnancy and never earlier than 21 weeks. This is probably due to the fact that the cysts become detectable only after there has been enough urine to distend the dysplastic tubules. The diagnosis of bilateral renal agenesis can be made by the 20th week of gestation. Several algorithms have been proposed to assist in the management of these patients [38]. In cases where ultrasound findings indicate a poor prognosis, the option to terminate the pregnancy should be discussed with the family.

Dominantly inherited renal adysplasia (IA1a, table 10-1)

Although most cases of renal agenesis and related abnormalities are sporadic, there are many reports of familial clustering. In 1973, Buchta et al. postulated the dominant inheritance of a gene for varying degrees of renal agenesis or severe dysplasia, which they termed hereditary renal adysplasia [40]. In a study of 199 siblings of patients with bilateral renal agenesis, Carter et al. found a recurrence rate of 3.5%, which is too high to be explained by multifactorial inheritance [41]. Silent renal abnormalities were found by ultrasound in 12% of 111 first-degree relatives of 41 infants who died because of bilateral renal agenesis or severe dysplasia without a recognizable specific malformation or disruption [42]. Abnormalities included unilateral renal agenesis, double ureter, hydronephrosis, multicystic kidney, and multiple renal cysts. Recently, McPherson et al. [43] described seven additional families with two or more cases of unilateral and/or bilateral renal agenesis or related abnormalities, reviewed the literature, and analyzed the pattern of inheritance in 90 kindreds [43]. The results suggested an autosomal dominant inheritance with a maximal penetrance of 80%–90% and with a minimal penetrance of approximately 50%. In families with strong evidence of dominant renal adysplasia, the penetrance for bilateral renal adysplasia in heterozygous was estimated to be 30%–40%. Because the recurrence rate for bilateral renal adysplasia was only 3.5% in the study by Carter et al. [41] and 4.4% in the study by Roodhoofd et

al. [42], it is clear that not all cases of bilateral renal adysplasia can be explained by inheritance of an autosomal dominant gene [44]. The phenotype has not been helpful in distinguishing dominantly inherited renal adysplasia from other causes of this condition. Nonrenal malformations are more common in the sporadic cases but can also occur in the dominantly inherited disease. On the basis of these observations, it is recommended that the parents of all infants with bilateral renal adysplasia, and possibly the parents of patients with unilateral renal agenesis, undergo renal evaluation regardless of any associated abnormalities in the proband. In families with evidence of dominant transmission of the gene, the empiric risk for severe bilateral disease in the offspring of heterozygotes is 15%–20%. Serial ultrasonography during pregnancy is strongly recommended. Due to incomplete penetrance, the risk for a child of a heterozygote to be heterozygote is significant (one sixth if penetrance is 80%). In families without a history of renal disease, the empiric risk figure of 3.5% for recurrence of bilateral renal adysplasia may be used [43].

Branchio-oto-renal syndrome (IA1b, table 10-1)

The branchio-oto-renal syndrome is another autosomal dominant disorder manifested by various combinations of preauricular pits, lateral cervical sinuses, cysts or fistulas, structural defects of the outer, middle, or inner ear, sensorineural, conductive, or mixed hearing loss, and renal abnormalities including renal agenesis and hypodysplastic and multicystic kidneys [45–47]. It is characterized by a high penetrance (almost all known carriers adequately examined show some manifestation) and variable expressivity (being harmless in a majority of cases). In 23% of the cases examined radiologically, there was severe agenesis or dysplasia, and in 43% mild asymptomatic dysplasia. If one assumes that all cases in which renal problems had not been reported did not have agenesis or severe dysplasia, the estimated risk for severe renal involvement is 6%.

It should be noted that preauricular pits are a very common minor anomaly occurring in about 1% of the Caucasian population, and most patients with preauricular pits do not have branchio-oto-renal syndrome. The estimated risk of a newborn child with preauricular pits to have profound hearing loss is 1 in 200, and to have branchio-oto-renal syndrome is 1 in 400.

Cryptophthalmos–syndactyly syndrome (IA1c, table 10-1)

Cryptophthalmos is a rare congenital malformation in which the eyelid folds are replaced by skin that passes from the forehead over the eye onto the cheek [48–49]. Syndactyly and other malformations are frequently associated. Unilateral or bilateral renal agenesis and related renal abnormalities occur in approximately 10% of the cases. The mode of inheritance is usually autosomal recessive. The likelihood of recurrence in subsequent pregnancies is therefore 25%.

Acrorenal field defect (IA1d, table 10-1)

The association of renal agenesis or related renal abnormalities and congenital malformations of hands and feet, such as syndactyly, polydactyly, and ectrodactyly, in the absence of other well-recognized malformations or dysfunctions, has been reported in a small number of patients [50, 51]. In one patient described to have acrorenal–mandibular syndrome, severe mandibular hypoplasia was also present [52]. An autosomal dominant or autosomal recessive pattern of inheritance has been suggested.

Skeletal dysplasias (IA1e, table 10-1)

Renal agenesis, cystic dysplasia, and related renal abnormalities have been reported in association with a variety of skeletal dysplasias with an autosomal recessive inheritance. The biochemical defects responsible for these disorders have not been identified, although it is known that the rhizomelic type of chondrodysplasia punctata is a peroxisomal disorder closely related to the Zellweger syndrome. Table 10-2 summarizes the main clinical features, prognosis, and prenatal diagnosis [53–76] of these conditions. Most of these diseases result in death at birth or during early childhood. Survival to adulthood occurs in chondroectodermal dysplasia, while patients with asphyxiating thoracic dystrophy who survive infancy develop renal insufficiency. Biliary dysgenesis and pancreatic dysplasia are prominent features in some of these diseases [77–82]. The renal involvement in some of these conditions includes features of both defects of metanephric differentiation and hereditary interstitial nephritis.

Other autosomal recessive metabolic dysplasias and multiple malformation syndromes (IA1f, table 10-1)

Cystic dysplasia and related renal abnormalities occur at varying rates in numerous autosomal recessive multiple malformation syndromes. A partial list of these conditions, along with their main clinical features, prognosis, and prenatal diagnosis, is shown in table 10-3 [79–99]. The renal involvement in cerebro-oculo-hepatorenal syndrome overlaps with that seen in the cystic diseases with inherited interstitial nephritis. Glutaric aciduria type II and the Zellweger syndrome deserve special comment.

Glutaric aciduria type II is an autosomal recessive disorder characterized clinically by metabolic acidosis, hypoglycemia, and excretion of a special group of organic acids in the urine. Patients with glutaric aciduria type II can be separated into two groups, those with deficiency in electron transfer flavoprotein and those with a deficiency in electron transfer flavoprotein dehydrogenase. Only those with the second type have associated congenital anomalies. The basic biochemical defect in the cerebro-hepatorenal syndrome of Zellweger has not yet been identified, but this syndrome is characterized by the absence of peroxisomes and defects in peroxisomal function, resulting in a

Table 10-2. Autosomal recessive skeletal dysplasias

	Phenotype	Renal involvement	Biliary dysgenesis	Pancreatic dysplasia	Prognosis	Prenatal diagnosis	Reference
Chondrodysplasia punctata (rhizomelic type)	Rhizomelic dwarfism Stippled epiphyses Joint contractures Characteristic face	Microcysts	No	No	Lethal in infancy	Amniotic fluid, amniocytes, chorinoic villi cells (?)	53, 54
Chondroectodermal dysplasia (Ellis–van Creveld syndrome)	Acromelic dwarfism Cone-shaped epiphyses Polydactyly Hypoplastic nails Dysplastic teeth Congenital heart disease	Agenesis, tubular microcysts, megaureter, interstitial nephritis	Yes	No	Death in childhood usual	Ultrasonography	53–57
Short-rib syndromes:	Dwarfism, short ribs, polydactyly, visceral abnormalities	Agenesis, hypoplasia, cystic dysplasia	Yes	Yes	Lethal in infancy	Ultrasound, fetoscopy	58–66
Type I (Majewski)	Short tibia, arhinencephalia						
Type II (Saldino–Noonan)	Metaphyseal/pelvic dysplasia	Tubular microcysts, interstitial nephritis	Yes	No	Respiratory failure in infancy; renal failure in childhood	Ultrasound	67–70
Type III (Naumoff)	Shortening of skull base						
Asphyxiating thoracic dysplasia (Jeune)	Rhizomelic dwarfism Short ribs Cone-shaped epiphysis Flat pelvis						

Acrodysplasia with retinitis pigmentosa (Saldino–Mainzer)	Cone-shaped epiphysis Retinitis pigmentosa Cerebellar truncal ataxia	Tubular microcysts, interstitial nephritis	?	?	Renal failure in childhood	—	71
Marden–Walker syndrome	Blepharophimosis Joint contractures Hypotonia Kyphoscoliosis Cleft palate	Microcysts	No	No	Failure to thrive	—	72
Roberts syndrome	Hypomelia Craniofacial dysmorphism Clitoral/penile enlargement Congenital heart disease	Cystic dysplasia, fused kidneys	Yes	No	Lethal in infancy or childhood	Ultrasound	73
Brachymesomelia-renal syndrome	Brachymesomelia Craniofacial abnormalities Congenital heart disease	glomerular microcysts	No	No	Lethal in infancy	—	74
Acrocephalopolysyndactyly dysplasia (Elejalde)	Organomegaly Craniosynostosis Polysyndactyly	Renal enlargement, tubular and glomerular microcysts	Yes	Yes	Lethal in infancy	—	75
Fryns syndrome	Distal limb hypoplasia Cleft palate/cleft lip Genital abnormalities	Cystic dysplasia, double ureters	No	No	Lethal in infancy	—	76

Table 10-3. Other autosomal recessive metabolic dysplasias and multiple malformation syndromes

	Phenotype	Renal involvement	Biliary dysgenesis	Pancreatic dysplasia	Prognosis	Prenatal diagnosis	Reference
Meckel (Simopoulos, Miranda)	Occipital meningoencephalocele, postaxial polydactyly	Agenesis, cystic dysplasia, glomerular cysts, fused kidneys, double ureters	Yes	No	Lethal in infancy or early childhood	Amniotic fluid (alpha-fetoprotein)	79–85
Renal–hepatic–pancreatic dysplasia (Ivemark)	Potter sequence, splenic abnormalities	Cystic dysplasia, glomerular cysts	Yes	Yes	Lethal in infancy	—	86, 87
Glutaric aciduria, type II	Craniofacial dysmorphism CNS dysgenesis Respiratory distress Metabolic acidosis Hypoglycemia "Sweaty feet" odor	Cystic dysplasia, tubular and glomerular microcysts	Yes	Yes	Lethal in infancy	Amniotic fluid amniocytes	88–91
Cerebro-hepatorenal syndrome (Zellweger)	Craniofacial dysmorphism CNS dysgenesis Hepatomegaly Stippled epiphyses	Glomerular and tubular microcysts, hydronephrosis, fused kidneys	Yes	No	Lethal in infancy	Amniocytes	92–98
Cerebro-oculo-hepatorenal syndrome (Arima)	Agenesis of cerebellar vermis, psychomotor retardation, Leber's congenital amaurosis	Cystic dysplasia, tubular microcysts, interstitial nephritis	Yes	No	Renal failure during childhood	—	99

number of biochemical abnormalities detectable in the tissues of these patients. The term *prenatal metabolic dysplasias* has been suggested to refer to a group of metabolic disorders, such as glutaric aciduria type II and the Zellweger syndrome, that result in similar defects of differentiation. Some of these conditions can be diagnosed prenatally by measurements on amniotic fluid or cultured amniocytes.

Chromosomal aberrations (IA2, table 10-1)

Characteristic phenotypes, often including the presence of renal cystic dysplasias and related abnormalities, occur in a variety of numerical or structural chromosomal aberrations [100]. Numerical aberrations are due to nondisjunction of sister chromatids at anaphase resulting in an aneuploid number of chromosomes. If nondisjunction occurs at a division of the zygote preceding gametogenesis, mosaicism with two or more cell lines with different numbers of chromosomes ensues. Chromosomal structural abnormalities include deletion, duplication, insertion, and translocation. Deletions are the structural abnormalities that most commonly result in abnormal phenotypes. Numerical chromosomal aberrations are usually sporadic, and the risk of recurrence is low. Exceptions are certain structural chromosomal rearrangements such as translocations that may increase the risk of nondisjunction during gametogenesis, and a mosaic state without phenotypic expression in a parent. The most common chromosomal aberrations with associated defects of metanephric differentiation include trisomies 21 (Down syndrome), 18 (Edwards), 13 (Patau), 8, 9, 7, and 2q; chromosome X monosomy (Turner); and 4p deletion (Wolf–Hirschhorn) [101–106].

Neural tube defects (IA3, table 10-1)

The association of neural tube defects and congenital renal abnormalities was recognized a long time ago, but the first detailed study was done by Roberts in 1961 [107]. Analysis of 140 cases of severe spina bifida revealed 25 cases (17.8%) with gross structural renal abnormalities. These include fused kidneys (9 cases), unilateral or bilateral renal agenesis or hypodysplasia (5 cases), and cystic kidneys (6 cases), in addition to ureteric, bladder, and urethral abnormalities. In a later study, Wilcock et al. [108] reported a 29% prevalence of gross renal abnormalities in children with meningomyelocele including cystic dysplasia in 6.9% of the patients. There is a relationship between the level of the neural lesion and the type of the abnormality [109]. Renal agenesis is associated with a sensory loss at the level of the T5-8 dermatomes, horseshoe kidneys are associated with the T9-L1, and duplications are associated predominantly with the sacral dermatomes. Neural tube defects are due to both genetic and environmental factors. The recurrence risk in subsequent pregnancies is estimated to be 3%–5% [110]. The levels of alpha-fetoprotein in the amniotic fluid are increased in open neural tube defects [111], making it possible to diagnose them by amniocentesis at 16 weeks of gestation.

Sporadic syndromes or associations (IA4, table 10-1)

Most defects of metanephric differentiation occur sporadically, either as isolated events or as a component of a number of syndromes or associations. The de Lange's syndrome consists of primordial growth deficiency, severe mental retardation, limb abnormalities, and a characteristic facies [112]. Significant upper urinary tract abnormalities, including renal agenesis and dysgenesis, have been associated with all three types of Klippel–Feil syndrome [113]: type I (fusion of all the cervical and upper thoracic vertebrae), type II (fusion of one or two pairs of cervical vertebrae), and type III (combination of type I or type II with lower thoracic or lumbar fusions). VATER is an acronym used to describe an association of anomalies including V = vertebral or vascular abnormalities; A = anal malformations; TE = tracheo-esophageal fistula, esophageal atresia; and R = radial or renal abnormalities [114].

Disruption syndromes including renal agenesis/dysplasia and associated renal abnormalities (IB, table 10-1)

The most common disruption syndromes that include renal agenesis/dysplasia and associated renal abnormalities are listed in table 10-1. Among them, maternal diabetes mellitus is probably the most common. The renal abnormalities may occur as isolated malformations or more frequently as part of multiple malformation syndromes, such as caudal regression syndrome and the VATER association [115, 116]. A strict control of diabetes mellitus before and during the pregnancy reduces the risk for these complications. Prenatal ultrasonography should be offered to the patients. It is presumed that many cases of the Williams syndrome are due to a maternal derangement in vitamin D or calcium metabolism [117, 118]. It has been suggested that populations of English and German origin may carry genes that predispose them to fetal hypercalcemic reactions to vitamin D [4].

RENAL CYSTIC DISEASES WITH HEREDITARY INTERSTITIAL NEPHRITIS (NEPHRONOPHTHISIS–MEDULLARY CYSTIC DISEASE COMPLEX (NMCDC))

Nephronophthisis and medullary cystic disease, initially described as two different diseases, are now considered synonymous [119]. At least two forms are recognized on the basis of inheritance and clinical presentation: a juvenile recessive form and an adult dominant form. In the recessive type, a subclinical form of the disease, manifested by a reduced urinary concentrating ability, has occasionally been documented in obligate heterozygotes. Sporadic presentations may represent new dominant mutations or recessive cases in families with a small number of descendants. Extrarenal abnormalities such as congenital hepatic fibrosis [120], retinitis pigmentosa (Senior syndrome) [121], skeletal dysplasias, and oculo-cerebro-hepatorenal dysplasias are frequently associated with autosomal recessive forms. Disorders of the kidney and retina may occur together or independently of one another. A renal disease consistent with nephronophthisis–medullary cystic disease complex (NMCDC) fre-

quently occurs in two autosomal recessive syndromes, the Laurence–Moon–Bardet–Biedl syndrome [122–124] and Alström's syndrome [125–126]. The Laurence–Moon–Bardet–Biedl syndrome is characterized by obesity, polydactyly, retinitis pigmentosa, mental retardation, and hypogenitalism. Renal involvement is very common and consists of tubulointerstitial nephritis and caliectasis, often with cystic spaces communicating with the collecting system. The Alström's syndrome is characterized by obesity, diabetes mellitus, retinitis pigmentosa, and nerve deafness. A sporadic case of renoretinal dysplasia with nuclear cataracts, sensorineural hearing loss, and diffuse glomerular cysts in addition to tubular cysts in the corticomedullary region has been reported [127]. The renal involvement in these forms of NMCDC with extrarenal manifestations overlaps considerably with the defects of metanephric differentiation discussed in the previous section.

In NMCDC, the kidneys are usually small, and contain multiple cysts located most commonly at the corticomedullary junction and along the medullary collecting ducts. However, cysts are not universally present and when present, are not necessarily confined to the medulla. For this reason, some authors object to the term *medullary cystic disease* [128]; others have suggested that nephronophthisis can lead to medullary cystic disease, and that the latter term should be used only when cysts are present [129]. Little is known about the pathogenesis of this disorder. Recently it has been proposed that NMCDC may be due to a primary defect in tubular basement membrane synthesis or to an inherited form of autoimmune nephritis. Cohen and Hoyer [130] have described morphological alterations in the basement membrane of all tubular segments; the glomeruli were spared. These alterations included extreme thinning and attenuation, layering, and thickening. As a result of these changes in the tubular wall, Tamm–Horsfall protein can escape into the interstitium and possibly contribute to the development of the interstitial nephritis and progressive renal damage. Kelly and Neilson [131] suggested that NMCDC is an inherited form of autoimmune interstitial nephritis. They based this hypothesis on the striking histological similarity between NMCDC and the spontaneous form of interstitial nephritis observed in kdkd mice, which is also inherited as an autosomal recessive trait.

At all ages, polyuria, nocturia, and thirst are the most common presenting symptoms of the NMCDC. In the juvenile recessive type, the onset of the disease occurs at a mean age of ten years, and the progression to renal failure is usually fast, over a three-year period. The adult dominant form also has an insidious onset at a mean age of 30 years and progresses rapidly to renal failure [123]. A late onset of the disease during the seventh and eighth decades of life has been observed in some families; therefore, NMCDC should not be discarded in the differential diagnosis of chronic renal failure even in the elderly [132, 133]. In some of these elderly patients, the clinical course has been prolonged. Hypertension may be present but is not a prominent feature of this disease. Sodium wasting is common. The urine sediment is characteristically

benign. Proteinuria is usually below 1 g per 24 hours, but nephrotic-range proteinuria of tubular origin has been reported in rare cases [134]. Detection of cysts by radiographic procedures is frequently unsuccessful because the cysts are usually small. The value of ultrasonography or computerized tomography for the detection of cysts is uncertain. In one study, medullary cysts were detected in 13 of 15 children with nephronophthisis and advanced uremia [139].

The treatment of medullary cystic diseases is only supportive. Because of the tendency to sodium wasting, volume contraction, and renal azotemia, unnecessary sodium restriction or use of diuretics should be avoided. If kidneys from living, related donors are to be used for transplantation, precautions should be taken to obtain them only from unaffected relatives, who should be ten or more years older than the affected child or sibling and should be subjected to meticulous diagnostic evaluation [136].

RENAL CYSTIC DISEASES WITH NORMAL METANEPHRIC DIFFERENTIATION AND HYPERPLASIA OF THE TUBULAR EPITHELIUM

Under this last category is included a heterogeneous group of disorders that needs to be considered in the differential diagnosis of bilateral cystic disease of the kidney. In these diseases, the cysts originate from completely formed nephrons, and there is no evidence of abnormal metanephric differentiation. Based on theoretical considerations, cyst growth in these diseases should be, at least to some extent, dependent on the production of new epithelial cells [137].

Autosomal recessive polycystic kidney disease (ARPKD) (IIIA, table 10-1)

ARPKD is an autosomal recessive disorder characterized by various combinations of bilateral cystic renal disease and hepatic fibrosis. The homozygous frequency at birth is unknown. A rough estimate may be between 1:10,000 and 1:40,000 [16,138]. It is controversial whether ARPKD is genetically heterogeneous or whether the different subtypes constitute a continuum within the spectrum of the disease. Four forms of ARPKD are recognized depending upon the age at onset and the percentage of renal tubules affected: perinatal, occurring at birth with 90% of the tubules affected; neonatal, recognized in the first month with 60% of tubules affected; infantile, between the ages of 3 and 6 months with 25% of tubules involved; juvenile, recognized after the first year of life with less than 10% of the tubules showing cystic dilatation [139]. There is an inverse relationship between the severity of the renal and hepatic lesions. Some authors argue that the different forms of ARPKD are genetically different, but their claim is not supported by the observation that members of the same family may present with different forms of the disease [140, 141]. The term *congenital hepatic fibrosis* is used to describe a developmental hepatic abnormality that is ordinarily associated but not restricted to ARPKD; it occurs also as an isolated abnormality and in association with renal dysplasia and hereditary tubulointerstitial nephritis [142].

Table 10-4. Differential diagnosis of ARPKD and ADPKD in children by imaging techniques*

	Ultrasound	Excretory urogram	Computerized tomography
ARPKD	Echogenicity Kidney > liver	"Streaky" pattern of contrast accumulation in enlarged collecting tubules	Retention of contrast in cortex, lacelike cysts in medulla
ADPKD	Macroscopic cysts	"Puddled" pattern if there is accumulation of contrast in collecting tubules	Contrast evenly distributed, cysts

*From [146].

Caroli's disease (nonobstructive intrahepatic biliary dilatation), which frequently overlaps with congenital hepatic fibrosis, can also occasionally be seen in association with ARPKD [143]. The association of ARPKD and Ehlers–Danlos syndrome has been reported in one patient [144].

ARPKD corresponds pathologically to the type I cystic kidneys in Osathanondh and Potter's classification. According to these authors, ARPKD kidneys result from the local gigantism of collecting tubules with excessive growth of the cells. There is no evidence of abnormal ampullary activity, as reflected by normal branching of the tubules and a normal number of nephrons. In contrast to autosomal dominant polycystic kidney disease (ADPKD), the cysts are elongated and of similar size, and located radially from the medulla to the cortex [14]. The hepatic abnormality in congenital hepatic fibrosis consists of enlarged and fibrotic portal areas with an apparent proliferation of bile ducts, absence of central bile ducts, hypoplasia of the portal vein branches, and sometimes prominent fibrosis around the central veins [142].

Initially thought to be always fatal, ARPKD has a variable clinical course, and some patients may have a prolonged survival [145]. Patients with the perinatal form present with Potter's sequence. Those with the neonatal type frequently develop hypertension and signs of chronic renal failure, while those with the infantile type often present with a combination of renal and hepatic disease. The hepatic involvement may become more apparent in these patients after successful renal transplantation. Portal hypertension is a common finding in patients with the juvenile form, but polycystic kidneys and chronic renal failure may become the main problem following successful portal shunting. The differentiation of ARPKD from autosomal dominant polycystic kidney disease (ADPKD) in infancy or childhood is not always easy [150, 151]. The affected parents of a child with early manifestations of ADPKD are frequently not aware of having the disease. A combination of radiologic examination of the child and both parents is essential for the diagnosis. According to Kaariainen et al. [146], it is usually possible to determine whether a child with a sporadic polycystic disease has ADPKD or ARPKD by excretory urography, ultrasonography, and computerized tomography. The diagnostic criteria are listed in table 10-4. Often, however, the diagnosis needs to be based

on kidney and, particularly, liver histology. Rapola and Kaariainen [147] found that cysts with low cuboidal epithelium constituted the main histologic finding in ARPKD, whereas in ADPKD the cyst epithelium was more variable and glomerular cysts were more frequent. More importantly, ARPKD is invariably associated with biliary dysgenesis, while histology of the liver in children with ADPKD is usually normal.

The prognosis for children with ARPKD diagnosed in infancy and surviving the neonatal period is not as dismal as initially thought [145]. Based on their experience, Cole et al. [145] recommend an aggressive approach to medical management of these children. Regular measurements of blood pressure and strict blood pressure control are necessary. Dialysis and renal transplantation are indicated when renal failure develops. A surgical portocaval shunt may be needed for severe portal hypertension.

Prenatal diagnosis of ARPKD by fetal ultrasonography during the second trimester of pregnancy is possible in some cases. However, a normal result does not ensure the absence of disease at birth, and the time in which the disease becomes detectable by sonography does not predict the severity of the disease at birth [148, 149]. Reports of high trehalase activity or alpha-fetoprotein levels in amniotic fluid have not been confirmed [150, 151].

Medullary sponge kidney (IIIB, table 10-1)

Medullary sponge kidney (MSK) or precalyceal canalicular ectasia is a common disorder characterized by tubular dilatation of the collecting ducts and cyst formation strictly confined to the medullary pyramids, especially to their inner, papillary portions [152]. It is usually regarded as a nonhereditary disease, but autosomal dominant inheritance has been suggested in several families [153, 154]. There have been several reports of MSK in patients with Ehlers–Danlos syndrome and in patients with hemihypertrophy [155–156].

In MSK, precalyceal canalicular ectasia may involve one or more renal papillae in one or both kidneys. These dilated tubules may be surrounded by normally appearing medullary interstitium or, in cases of more prominent cystic disease, inflammatory cell infiltration or interstitial fibrosis [157]. The renal size is usually normal or slightly enlarged. The rarity of reported cases of this disorder among children favors the interpretation that this is an acquired disease. Progression of the tubular ectasia and development of tubal dilatation and medullary cysts have been documented in some patients.

MSK is usually a benign disorder that may remain asymptomatic and undetected for life [158–160]. Impairment of tubular functions, such as a mild concentration defect, a reduced capacity to lower the urine pH after administration of ammonium chloride as compared to controls, and possibly a low maximal excretion of potassium after short-term intravenous potassium chloride loading may be documented in these patients [161, 162]. As many as 30%–40% may fit the definition of incomplete distal renal tubular acidosis.

The complete form of distal renal tubular acidosis with hypokalemia occurs less frequently [163]. The major complication of MSK kidney is the deposition of gross or microscopic calculi in the dilated or cystic tubules; these calculi often are responsible for the development of gross or microscopic hematuria and episodes of renal colic. The association between precaliceal tubular ectasia and nephrolithiasis is well documented [160, 164]. Patients with MSK and nephrolithiasis have a higher rate of stone formation than other patients with idiopathic nephrolithiasis. The major factor responsible for the formation of stones in these patients is presumed to be stagnation of the urine in the dilated or cystic tubules. Other metabolic factors, such as impaired acidification mechanisms and hypercalciuria, may also play a role. Hypercalciuria and hyperparathyroidism have been frequently reported in patients with MSK and nephrolithiasis [165]. The hypothesis that these patients have renal leak hypercalciuria resulting in parathyroid overactivity and eventually autonomous hyperparathyroidism remains unproven [166]. In fact, absorptive hypercalciuria or parathyroid adenomas rather than hyperplasia, have been found in most patients with MSK who had either hypercalciuria or hyperparathyroidism. Although in a small series of selected patients the prevalence of parathyroid hyperactivity has been high, the frequency of hyperparathyroidism in a larger number of patients with MSK (approximately 5%) was not different from that observed in a large unselected group of patients with calcium nephrolithiasis. Similarly, the frequency of hypercalciuria in patients with calcium nephrolithiasis, with or without MSK, was not different. Patients with MSK may have a greater anatomic propensity to form stones, but the evaluation and treatment of any possible metabolic defect should not be different from other stone formers [167]. The stones are most commonly calcium oxalate or calcium phosphate. Although the diagnosis of MSK is frequently made during the evaluation for a urinary tract infection, the rate of infection is not different in calcium stone formers with or without MSK. Hypertension is not more frequent than in the general population, and the renal function remains normal except in rare patients with chronic pyelonephritis resulting from recurrent episodes of obstruction, infection, and multiple surgeries.

The diagnosis of MSK is made by excretory urography that characteristically reveals the dilated collecting tubules. Medullary sponge kidneys are commonly bilateral, but they may be unilateral in up to one fourth of the cases. The degree of tubular dilatation is highly variable and the wide range of reported frequencies of this abnormality is in part due to the variable degrees of collecting duct dilatation that different observers have decided to consider abnormal. Urographic degrees of tubular dilatation include a papillary blush (which is the mildest form of MSK), linear striations consistent with tubular dilatation, and different cystic forms that have been described as a bunch of flowers or cluster of grapes. Mild cases of precaliceal ectasia can easily be overlooked if the excretory urogram is not of high quality, clearly outlining most caliceal fornices, or if the images are obscured by overlying bowel. A

definitive diagnosis of MSK can be made when the dilated collecting ducts are visualized on early and delayed films without the use of compression and in the absence of ureteral obstruction. Deposition of calcium salts within these dilated tubules may give the radiographic appearance of renal calculi or nephrocalcinosis. The distribution of the renal calculi in these patients is characteristic, in clusters fanning away from the calyx. In rare cases, MSK can mimic the urography appearance of ADPKD [168]. In these cases, computerized tomography, ultrasonography, and arteriography help to distinguish these two lesions, clearly showing that the cortical layer is free of cysts, except where large medullary cysts protrude through the cortex to the surface. Precaliceal canalicular ectasia can be observed frequently in patients with ADPKD [169–172].

There is no specific treatment for MSK. The treatment of nephrolithiasis and urinary tract infection, when present, is the same as it would be in the general population. Thiazides and inorganic phosphates have been found to be effective in preventing stones in these patients. Extensive or repeated unnecessary investigations for hematuria should be avoided.

Autosomal dominant polycystic kidney disease (IIIC, table 10-1)

ADPKD is a multisystem disease with high penetrance that mainly affects the kidneys. It may present at various ages, and has a wide spectrum of clinical severity. The criteria for diagnosis are the presence of cystic and enlarged kidneys, a family history consistent with an autosomal dominant inheritance, and the exclusion of other disorders associated with cystic kidneys. It has a minimal prevalence (lifetime risk of diagnosis of 1 in 1000), and it is estimated that 10% of all cases of end-stage renal failure can be attributed to this disease [173].

In 1985, Reeders et al. [174] localized the mutation for ADPKD to the short arm of chromosome 16 by demonstrating genetic linkage with the alpha-chain of human hemoglobin and with phosphoglycolate phosphatase. Initially, the linkage between the alpha-globin locus and ADPKD was found in all the families studied, with and without a history of renal failure, with a recombination frequency of approximately 5% [175–178]. These studies supported the existence of a single locus on chromosome 16 responsible for the disorder. However, the apparent lack of linkage in a few families suggests the possibility of genetic heterogeneity. In certain families, clinical manifestations of ADPKD tend to occur early in life [179–182]. Possible reasons are homozygosity for ADPKD, for which there is no evidence; compound heterozygosity for ADPKD and a different renal cystic disease, such as ARPKD; modifying alleles at the same gene locus; and heterogeneity due to multiple PKD alleles [179–182]. The early age at onset and extreme severity of the clinical manifestations may also be the result of variable expressivity, constituting the opposite end of the spectrum from those cases that remain asymptomatic

Table 10-5. Minimal diagnostic criteria for tuberous sclerosis*

One finding	Two findings
Facial agiofibroma or periungual fibroma (by biopsy)	Infantile spasms
Cortical tuber, subependymal nodule, or giant-cell astrocytoma	Hypomelanotic macules
Multiple retinal hamartomas	Shagreen patch
Combination of renal angiomyolipomas and cysts	Single retinal hamartoma
	Subependymal or cortical calcifications (CT scan)
	Bilateral renal tumors (angiomyolipomas or cysts)
	Cardiac rhabdomyoma
	Family histor of tuberous sclerosis in a first-degree relative

*According to Gomez et al. From [195].

throughout life. Associations of ADPKD and other inherited diseases have been reported in some families. These associations are more likely fortuitous and include familial polyposis of the colon, Peutz–Jeghers syndrome, hypertrophic pyloric stenosis, myotonic dystrophy, lattice corneal dystrophy, Darier's disease, hereditary spherocytosis, carotid glomus tumor, $alpha_1$-antitrypsin deficiency, and Marfan's syndrome [183–191]. ADPKD has also been observed in association with hemihypertrophy [192]. The clinical and genetic aspects of ADPKD are discussed in more detail elsewhere in this volume.

Tuberous sclerosis (IIID, table 10-1)

Tuberous sclerosis is an autosomal dominant disease with high penetrance, extremely variable expression, and a high rate of spontaneous mutation [193–195]. It may affect the central nervous system (cortical tubers, subependymal nodules, and giant-cell tumors), skin (facial angiofibroma or adenoma sebaceum, fibrous plaques, peri- or subungual fibromas, hypomelanotic macules, and shagreen patches), retina (hamartoma), kidneys (angiomyolipomas and cysts), heart (rhabdomyoma), and lungs. Other organs may also be involved, but the lesions are usually asymptomatic. Because the clinical features are so variable, minimal diagnostic criteria have been established (table 10-5). Few cases of nonpenetrance in obligatory carriers of the disease have been reported [196]. The possibility that these apparent cases of nonpenetrance might reflect germ-line mosaicism has been suggested [197]. Assignment of the gene for tuberous sclerosis to the distal long arm of chromosome 9 was first suggested by demonstration of linkage of tuberous sclerosis to the ABO blood group [198]. According to other authors, however, if tuberous sclerosis is linked to the ABO blood group, the recombination fraction is larger than suggested in the initial reports [199,200]. Recently, the assignment of tuberous sclerosis to chromosome 9 has been supported by the demonstration of linkage to a DNA

polymorphism detected by pSA-19, which is a fragment of the transforming sequence v-*abl* first discovered in the Abelson strain of murine leukemia virus [201]. The oncogene c-*abl* is the human homologue of v-*abl* and has been localized in the long arm of chromosome 9.

Two different varieties of renal lesions are found in association or independently in patients with tuberous sclerosis: angiomyolipomas and renal cysts [202–209]. A study by Stillwell et al. [203] revealed that 47% of clinical cases seen at the Mayo Clinic had angiomyolipomas, 18% had cysts, and 12% had both lesions, while in autopsy cases at the same institution 73% had angiomyolipomas, 53% had renal cysts, and 27% had both lesions. The occurrence of both lesions in the same patient is diagnostic of tuberous sclerosis. The angiomyolipomas vary in size from a few millimeters to several centimeters in diameter and are composed of fat cells with a foamy appearance mixed with smooth muscle cells that surround thick-walled arterioles. The cysts are lined with characteristic hypertrophic and hyperplastic, strongly eosinophilic cells that contain large hyperchromatic nuclei with occasional mitotic figures [202]. Several patients with tuberous sclerosis and renal-cell carcinomas have been reported [209]. In contrast to patients with renal carcinoma without tuberous sclerosis, the patients with tuberous sclerosis and renal carcinoma have a female predominance, frequent bilateral involvement, and usually a good prognosis following surgery.

Patients with angiomyolipomas may be asymptomatic or may present with gross hematuria, lumbar or flank pain, or fever. Retroperitoneal hemorrhage is a potentially fatal complication. Patients with multiple angiomyolipomas may develop renal failure, but this is more common among patients with severe cystic disease. Severe diffuse cystic disease may be macroscopically indistinguishable from ADPKD [204–206]. Patients with severe diffuse bilateral cystic involvement often present in childhood with bilateral renal masses and hypertension even before developing other manifestations of tuberous sclerosis. Usually renal failure does not develop until the second or third decade of life. A search for additional manifestations of tuberous sclerosis is warranted in young patients presenting with polycystic kidney disease. Differentiation of an angiomyolipoma from renal cell carcinoma by excretory urography and angiography is difficult. Computerized tomography, however, demonstrates the fat in the angiomyolipoma and clearly separates these two lesions.

Angiomyolipomas require no treatment except for life-threatening bleeding, which may require arterial embolization or segmental nephrectomy. The value of cyst decompression to protect renal parenchyma remains uncertain. Treatment of renal failure is not different from that of ADPKD.

To permit appropriate genetic counseling, phenotypically normal parents of an affected child, if still of reproductive age, should undergo a thorough examination. This should include a meticulous examination of the skin with a Wood lamp, ophthalmologic examination with dilatation of pupils, cranial computerized tomography with contrast, and renal ultrasound. Some authors

Table 10-6. Diagnostic criteria for von Hippel–Lindau disease*

CNS or retinal hemangioblastoma
or
CNS or retinal hemangioblastoma plus one of the following:
- Renal, pancreatic, hepatic, or epididymal cysts
- Pheochromocytoma
- Renal cancer

or
Definite family history plus one of the following:
- CNS or retinal hemangioblastoma
- Renal, pancreatic, or epididymal cysts
- Pheochromocytma
- Renal cancer

*From [215].

also suggest examination of the teeth for pitted enamel hypoplasia and x-rays of the skull, hands, and feet to search for bony sclerosis and bone cysts [210, 211]. If all of these examinations are negative, one parent can still be affected (nonpenetrance or germ-line mosaicism), but the likelihood is very low. When one parent is affected, both parents should be informed that there is a 50% probability of having affected children. Prenatal diagnosis of tuberous sclerosis has been made in some cases by identifying cardiac rhabdomyomas echocardiographically during the late mid- and the third trimester [212, 213]. In one case, the diagnosis was confirmed by prenatal demonstration of a large cerebral tumor. DNA analysis has been used to exclude the diagnosis of tuberous sclerosis in one pregnancy at risk [214]. Additional studies, however, are needed before linkage analysis for prenatal diagnoses of tuberous sclerosis can become routine.

Von Hippel–Lindau disease (IIIE, table 10-1)

Von Hippel–Lindau disease is an autosomal dominant disorder with high penetrance and variable expression [215–217]. It affects most frequently the cerebellum, medulla oblongata, and the spinal cord (hemangioblastomas), retina (angiomatosis), kidneys (cysts, hemangiomas, adenomas, and carcinomas), pancreas (cysts and, rarely, tumors), and adrenal glands (pheochromocytoma). Criteria for diagnosis are listed in table 10-6.

Renal lesions of von Hippel–Lindau disease include cysts, hemangiomas, benign adenomas, and most importantly, malignant renal-cell carcinomas [218, 219]. The cysts are usually multiple, limited in number, and lined by an epithelium that is often irregularly hyperplastic due to mural nodules of clear carcinoma cells. Occasionally, the kidneys may be diffusely cystic, resembling ADPKD; some of the patients described in the literature as having renal-cell carcinoma and ADPKD probably had von Hippel-Lindau disease [220, 221]. In autopsy series, nearly one half of the patients with von Hippel–Lindau disease have renal-cell carcinomas. In contrast to the renal carcinomas observed

in the general population, they are frequently multicentric and bilateral and there is no male predominance. Recently, a proximal 3p deletion was identified in renal clear-carcinoma cells from a patient with von Hippel–Lindau disease [222]. It is possible that the initiation of neoplasia under these circumstances results from the loss of a regulatory mechanism.

The renal cysts are asymptomatic and usually require no treatment, but renal failure can develop in patients with diffuse cystic involvement. If the patient requires dialysis, nephrectomy should be performed because of the risk of carcinoma. Renal-cell carcinomas are the major cause of morbidity and are second only to hemangioblastoma of the CNS as a cause of death in these patients. When a carcinoma is removed by surgery, an effort should be made to spare as much renal tissue as possible because bilateral and multiple lesions are apt to be present or to develop subsequently [218, 219]. Early diagnosis of the lesions posing a health risk to the patient is essential. Patients diagnosed as having von Hippel–Lindau disease need annually a physical and ophthalmologic examination and a computerized tomography of the abdomen [223]. Annual determinations of urinary metanephrines and biannual computerized tomography of the head may also be reasonable. All first-degree relatives should have physical and ophthalmologic examinations and computerized tomography of the head and the abdomen. Any at-risk persons should be rescreened prior to reaching the reproductive age. Prenatal diagnosis of von Hippel–Lindau disease is not possible.

Neurofibromatosis (IIIF, table 10-1)

Neurofibromatosis is a common disorder with an estimated prevalence of 1 in 3000 and characterized by the presence of multiple neurofibromas and café-au-lait spots. It has become apparent that there is more than one form of neurofibromatosis. Iris Lisch nodules, optic gliomas and other intracranial astrocytomas, and pseudarthroses appear to be specific for neurofibromatosis-1, which accounts for approximately 85% of the cases. The acoustic form of neurofibromatosis is characterized by the presence of intracranial and spinal schwannomas and meningiomas. Other forms of neurofibromatosis are not well characterized [224]. Neurofibromatosis-1 is linked to the gene for the nerve growth factor receptor on the long arm of chromosome 17 [225, 226]. The gene for the acoustic form of neurofibromatosis has been localized to chromosome 22 [227]. Renal cystic disease is not a common feature of neurofibromatosis. There have been only a few reports of neurofibromatosis and adult polycystic kidney disease in one of the cases associated with renal-cell carcinoma [228, 229]. These cases could represent chance associations.

Oro-facio-digital syndrome type I (IIIG, table 10-1)

Oro-facio-digital syndrome type I is a rare sex-linked dominant disorder [230, 235]. Affected males are stillborn, and as many as one third of females die in their first year of life. The syndrome is characterized by a combination of

abnormalities of the oral cavity (hyperplastic frenula, cleft tongue, and often cleft palate or lip and malposed teeth), face (broad nasal root with hypoplasia of nasal alae and malar bone), and digits (brachy; syn; clino; campto; polydactyly); mental retardation and tremor can be present in up to 30%–50% of the patients. Liver cysts are usually absent. Renal failure may ensue at any time during the second through the seventh decade of life. Because most patients with oro-facio-digital syndrome type I come to medical attention in early childhood for surgical repair of the external malformations, their kidney involvement may not be apparent at the time of initial diagnosis. Recognition of the syndrome has important genetic implications. In contrast to ADPKD, males and the phenotypically normal females are not at risk for kidney disease. Only the female relatives of an index case are at risk of kidney disease. Features of this syndrome should be looked for in a family where only females have polycystic kidneys. Conversely, females diagnosed as having oro-facio-digital syndrome type I should be investigated for renal functional and structural abnormalities.

Glomerulocystic kidney disease (IIIH, table 10-1)

Cystic dilatations of Bowman's space can occur in association with many conditions causing abnormal metanephric differentiation (i.e., urinary tract obstruction) and in a variety of cystic diseases such as ADPKD, tuberous sclerosis, and oro-facio-digital syndrome type I. The familial hypoplastic glomerular cystic kidneys described by Rizzoni et al. [236] probably belong to the first category. The term glomerulocystic kidney disease refers to a group of renal cystic diseases characterized by the predominance of glomerular cysts, absence of or minimal tubular involvement, and by the lack of urinary tract obstruction, renal dysplasia, or evidence of a recognizable cystic disease or malformation-disruption syndrome [237–248]. Most initially described cases that met this definition were infants or young children, without a family history of renal disease, presenting with enlarged kidneys or variable degrees of renal insufficiency. The disease was not recognized until recently in adults [247, 248], possibly because adults with multiple renal cysts are assumed to have more common conditions, such as simple renal cysts or ADPDK, and are not subjected to renal biopsy. In the adult cases, inheritance appears to be dominant without sex linkage.

Glomerulocystic kidney disease is characterized histologically by cystic dilatation of Bowman's spaces, forming numerous cysts in the renal cortex ranging in size from microscopic to as large as 7 cm in diameter. Some authors have described papillary hyperplasia of the lining epithelium, but most cysts are lined by a single layer of flattened to cuboidal epithelium. Medullary interstitial inflammation and fibrosis have been noted in some cases. The mechanism leading to the formation of glomerular cysts is unknown. Intrauterine exposure to drugs such as phenacetin and other environmental or genetic factors causing intrarenal obstruction have been suggested.

The natural history of glomerulocystic kidney disease is not well known.

Very few children with this disease have had prolonged follow-up. Those who survive childhood probably have stable renal function for many years. The clinical presentation of glomerulocystic kidney disease in the adult may resemble that of ADPKD. Screening of the first-degree relatives by ultrasonography seems reasonable. Because of the limited information available, genetic counseling cannot be more precise.

Simple cysts (IIII, table 10-1)

Simple cysts are common particularly in elderly patients [249, 250]. Over 50% of people over 50 years old have at least one cyst on postmortem examination. Twenty-four percent of patients over the age of 40 have cysts detectable by computerized tomography of the abdomen obtained for reasons unrelated to the kidney. The clinical significance of cysts resides in the fact that they need to be differentiated from more serious renal diseases.

Clinical and pathologic studies suggest that most, if not all, simple renal cysts are acquired, but the contribution of genetic factors to the development of simple cysts has not been studied. Simple renal cysts are usually lined by a single layer of epithelial cells and filled with a clear, serous fluid [250]. They are usually small and grow slowly, but huge cysts of up to 30 cm in diameter have been described. The inner surface of these cysts is glistening and usually smooth, but some cysts may be trabeculated by partial septa that divide the cavity into broadly interconnecting locules. These septated simple cysts should not be confused with multilocular cysts. Several hypotheses have been proposed to explain their pathogenesis. Animal studies have suggested that tubular obstruction and ischemia might play a role. Interstitial fibrosis, which increases with age, may cause tubular obstruction, which may contribute to the development of the cysts [251]. Microdissection studies indicate that localized defects in the tubular wall may lead to formation of a diverticulum that progresses to a simple cyst. Diverticuli of distal convoluted tubules can be frequently found by microdissection techniques after the age of 20 years, and increase in number with age [252].

The cysts are usually asymptomatic, being discovered at the time of a nephrourologic evaluation for some unrelated problem [253]. It is, therefore, important that the presence of these cysts does not distract from the diagnosis of more important intrarenal or extrarenal lesions. Large renal cysts may cause abdominal or flank discomfort, often described as a sensation of weight or a dull ache. More frequently, however, this pain can be explained by a coexisting abnormality such as nephrolithiasis. Rare cases of gross hematuria due to vascular erosion by an enlarging cyst have been documented. In the majority of patients, however, hematuria is due to another cause. When the simple cysts lie at or near the hilus, a urographic pattern of caliceal obstruction or hydronephrosis is frequently found. In most but not all cases, these apparent obstructive changes are of no functional significance. A dynamic hippuran/

DTPA radioactive renal scan before and after administration of furosemide can help to assess the degree of obstruction. Rare cases of renin-dependent hypertension caused by solitary intrarenal simple cysts have been described. The proposed mechanism is arterial compression by the cyst causing segmental renal ischemia. Infection is a rare but dramatic complication of a renal cyst [254]. The patient presents usually with high fever, flank pain and tenderness, and frequently a sympathetic pleural effusion. Most patients are females and the most common pathogen is *Escherichia coli*. Urine cultures are frequently negative. By far the most common diagnostic problem raised by the detection of a simple cyst is its differentiation from a renal-cell carcinoma. Because the appearance of a renal mass on the excretory urogram alone never excludes a malignancy, ultrasonography, computerized tomography, or arteriography is commonly required to characterize the lesion [255, 256]. Acceptance of definite criteria for the diagnosis of a simple cyst by ultrasonography or computerized tomography has resulted in a dramatic decline in the number of angiograms performed for differential diagnosis of renal masses. Angiography is still helpful, especially in patients with complex, multiple renal masses such as multiple cysts and possible solid renal tumors.

Improvements in the diagnostic techniques have reduced substantially the indications for surgery in the management of benign simple cysts. Surgery is indicated only in the rare cases where the diagnosis is still in doubt, in the rare complicated cysts that cannot be adequately treated percutaneously, and in symptomatic cysts that recur rapidly after percutaneous drainage.

Simple cysts are not inherited. Occasionally simple cysts may be very numerous and cause parenchymal and pyelocaliceal distortion. When both kidneys are extensively and diffusely involved, differentiation from ADPKD may be difficult. Because of the obvious implications, it is important to avoid the diagnosis of ADPKD unless a familial history consistent with autosomal dominant transmission can be documented.

Acquired cystic disease of the kidneys (IIIJ, table 10-1)

The term *acquired cystic disease of the kidneys* has been used to describe the cystic degeneration of the renal parenchyma that occurs in end-stage kidneys [257, 258]. It appears that these cystic changes can start prior to the initiation of dialysis and that they develop regardless of the type of dialysis being used. This phenomenon is, therefore, likely to be related to the uremic state rather than to be a consequence of the dialysis procedure. The fact that very often these cysts regress following successful renal transplantation supports this assumption.

NEOPLASTIC CYSTS

Cysts can be found in a variety of neoplastic diseases of the kidney [259–261], but they are a constant feature only in the multilocular cystic nephroma.

Multilocular cysts (IVA1, table 10-1)

Multilocular renal cysts constitute a well-defined entity consisting of a well-circumscribed, encapsulated renal mass composed of multiple noncommunicating cysts of varying size [262–266]. Unlike many other types of cystic kidneys, the predisposition towards development of multilocular cysts is not transmitted genetically. There are no associated malformations of the urinary tract or other organs.

Two histologic types of multilocular cysts have been described. The first type of multilocular cysts is found in infants and young children only; the septa in this type contain variable amounts of embryonic tissue composed of clusters of small cells with varying degrees of differentiation into structures such as tubules, glomeruli, and mesenchymal tissues, including smooth and striated muscle. The second type of multilocular cysts is most frequently found in adults; the septa in this type are composed of fibrous tissue. In both types, no fully developed nephrons or segments of nephrons can be found in the septa of the cysts, while the remaining kidney tissue outside the cysts is normal. The locules of the cysts are lined with flattened or plump, cytologically atypical epithelial cells. In some cases, proliferation of these cells has been interpreted as evidence of their neoplastic nature and occasionally histologic evidence of renal adenocarcinoma has been found. The biologic course of these tumors, however, is usually benign. Only a few cases have been reported in which development of a multilocular cyst has occurred in a kidney found previously to be normal by excretory urography.

Multilocular cysts are less rare than initially suspected. Approximately half of the cases reported have occurred in children and half in adults. In adults, the age at diagnosis ranged from 18 to 72 years, with a peak incidence in the sixth decade. Multilocular cysts are typically solitary and unilateral. Bilateral multilocular renal cysts are very rare and have been described mainly in children. The presenting symptoms are usually an abdominal mass, pain, or hematuria. Multilocular cysts are usually first detected on excretory urography, which generally cannot distinguish them from other renal masses. By ultrasonography, multilocular cysts are complex masses with well-defined cysts mixed with highly echogenic stroma. The role of computerized tomography in the diagnosis of these lesions has not been evaluated thoroughly. On angiography these lesions are generally avascular or sparsely vascular, rarely being moderately vascular or hypervascular. The angiographic appearance is, therefore, insufficient to rule out a renal-cell carcinoma. Cyst puncture reveals clear fluid with benign cytology. Only one or a few cysts are filled with contrast if this is instilled. If technically possible, partial nephrectomy is the treatment of choice for multilocular cysts. The occurrence of renal-cell carcinoma in multilocular cysts is not frequent enough to justify routine nephrectomy in these cases. However, tissue sampling for histologic studies and frozen sections should be done at the time of surgery to rule out the presence of a renal adenocarcinoma, which would necessitate complete nephrectomy. A preoperative diagnosis of

multilocular cysts free of renal adenocarcinoma is difficult to establish. On the other hand, atypical hyperplasias in multilocular cysts may on occasion be erroneously interpreted as low-grade adenocarcinomas. In any case, the biological course of multilocular cysts is usually benign, even in those cases with associated renal-cell carcinoma. Local or metastatic recurrences following nephrectomy for a multilocular cyst with adenocarcinoma are rare.

INFLAMMATORY CYSTS

Medullary cavities resulting from analgesic-related papillary necrosis or from mycobacterial or other bacterial infections sometimes need to be considered in the differential diagnosis of medullary sponge kidney and caliceal diverticula. A rare cystic renal disorder that has been known for centuries is renal infection by *Echinococcus granulosus* (hydatid cysts). Detailed discussion of these disorders is not within the scope of this chapter.

RENAL CYSTS OF NONTUBULAR ORIGIN

Cystic disease of the renal sinus (VIA, table 10-1)

Less is known about the cystic disorders involving the renal sinus than about those involving the renal parenchyma. The differential diagnosis of mass-occupying lesions in the area of the renal sinus is difficult, and many different processes may look the same on excretory urography. Identification of different types of lesions has become much easier with techniques such as computerized tomography and magnetic resonance imaging. The cystic disorders of the renal sinus are benign conditions, and they should not be confused with other more serious mass-occupying lesions of the renal pelvis or renal parenchyma. Two types of cystic lesions have been described in this area: the hilus cysts and the parapelvic cysts.

Hilus cysts have only been identified at autopsy and have been thought to be due to regressive changes in the fat tissue of the renal sinus, especially in kidneys with abundant fat in the renal sinus associated with renal atrophy. The cysts result from fluid replacement of adipose tissue that undergoes regressive changes owing to localized vascular disease and/or atrophy due to recent wasting. The wall of such a cyst is lined by a single layer of flattened mesenchymal cells, and the cystic fluid is clear and contains abundant lipid droplets [267, 268]. The parapelvic cysts are of lymphatic origin. The wall of the cyst is very thin and lined by flat endothelial cells. The composition of the cystic fluid resembles that of lymph. The lymphatic origin of the parapelvic cysts is not only supported by the structure of the wall of the cyst and the composition of the cystic fluid, but also by the location of the cysts and the good correspondence in many cases between the observed number of cysts and the normal number of renal hilar lymphatic channels. The mechanism responsible for the dilatation of these lymphatic channels is not known [269, 270].

Recently, it has become recognized that parapelvic cysts are multiple and bilateral much more frequently than initially thought, although in clinical and

surgical series only the larger cysts are recognized and the smaller cysts are overlooked [271]. Parapelvic cysts are in direct contact with the extrarenal pelvic surface and/or extend into the renal sinus, distorting the caliceal infundibuli and calices. The kidney may appear slightly enlarged, but the enlargement is exclusively due to the expansion of the renal sinus, whereas the area of the renal parenchyma per se remains normal.

The parapelvic cysts are most frequently diagnosed after the fourth decade of life. They are usually discovered in the course of evaluations for conditions such as urinary tract infections, nephrolithiasis, hypertension, and prostatism. Despite considerable distortion of the calices and infundibuli, the pressure in these lymphatic cysts is low and not likely to result in significant functional obstruction. Indeed, renal function in patients with bilateral multiple parapelvic cysts is usually normal. Occasionally, parapelvic cysts are the only finding in the course of evaluation for otherwise unexplained lumbar or flank pain.

The diagnosis of parapelvic cysts can be made by excretory urography with nephrotomography. The most common abnormality is the deformity caused by the elongation of the infundibuli extending into delicately lined calices. Contrary to simple renal cysts, there is no sharp interface between the contrast-laden parenchyma and the cysts on the nephrotomogram. The renal sinus fat displaced by the expanding parapelvic cyst may be detectable as a radiolucent halo, which has been called the peripheral fat sign. Unlike the cysts of medullary sponge kidney and communicating pelvic diverticulae, the parapelvic cysts never became opacified by contrast media. The introduction of computerized tomography and ultrasonography has facilitated the differentiation of parapelvic cysts from other benign conditions such as renal sinus lipomatosis, as well as from more serious diseases such as neoplasms of the renal pelvis and kidney or ADPKD. Bilateral renal parapelvic cysts causing distortion of the infundibuli and the calices and enlargement of the kidney have occasionally been mistaken for ADPKD. With the use of computerized tomography, the distinction of these two conditions is easy; in the cystic disease of the renal sinus or multiple parapelvic cysts, the cysts are confined to the renal sinus, although in some cases they can herniate out of the hilar space into the interstitium of the renal parenchyma. The therapeutic approach to parapelvic cysts should be conservative.

Pelvicaliceal Diverticulae (VIB, table 10-1)

These are cystic cavities that contain urine and are lined by transitional epithelium [272]. They may be contained in the renal parenchyma and be linked to the fornix of a minor calyx by a narrow isthmus (caliceal diverticulum) or be extrarenal and in direct communication with the renal pelvis (pelvic diverticulum). It is uncertain whether these diverticulae are of congenital or acquired origin. They are usually better demonstrated by retrograde pyelography than by excretory urography. They are usually asymptomatic

unless complicated by nephrolithiasis or infection. The frequency of stone formation in caliceal diverticulae has been reported to be between 10% and 50%. Surgical intervention is indicated only when conservative management of this complication fails.

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11. A MOLECULAR APPROACH TO AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the commonest genetic diseases of man. It is estimated that approximately one per 1000 of the U.S. population carries a mutation that leads to this disease. Although the most serious clinical manifestation of ADPKD is renal failure, which usually presents in middle life, there is substantial earlier morbidity from urinary infection, flank pain, hematuria, and hypertension. In addition to the mortality from renal failure, there is a significant loss of life from subarachnoid hemorrhage, which affects about 15% of ADPKD sufferers, many of whom are otherwise asymptomatic [1]. ADPKD also imposes a considerable burden on family and community resources, since it currently accounts for approximately 10% of all chronic renal dialysis and transplantation requirements in the U.S., at a cost of over $200,000,000 annually. No specific therapy is known to retard the development of cysts or to affect the rate of progression to end-stage renal failure. Furthermore, our understanding of the way in which the genetic mutation leads to the formation of cysts remains limited: none of the models postulated to explain the pathophysiology of this disorder has adequately explained how the genetic defect leads to the cellular and subsequent clinical pathology. Moreover, ADPKD is notable for its marked prognostic variability and phenotypic heterogeneity; there is variability in the age of presentation of the first symptoms, the rate of loss of GFR, prevalence of extra-renal cysts, and the incidence of subarachnoid hemorrhage. The additional observation that, in some individuals, the inheri-

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tance of the renal morphology characteristic of ADPKD is dissociated from the development of renal failure highlights the complex relationship between the development of cysts and the ensuing functional impairment [2–4]. The aim of our work is to study the molecular genetics of ADPKD as a means to answer these questions.

Until recently the only way to investigate the molecular basis of an inherited disorder was to look for abnormalities in either the structure or quantity of gene products (proteins). The successful application of this approach led to much of our current understanding of the molecular pathology of the thalassemia syndromes, for example. However, there are many inherited disorders of man, such as ADPKD, whose underlying biochemical defect is unknown. A novel approach, termed *reverse genetics* [5], has been proposed as a method of arriving at an understanding of the molecular pathology of a genetic disease without the prior identification of a specific protein abnormality (figure 11-1). In the reverse genetic approach, the mutant gene is first localized to a chromosomal region by comparing the segregation of the disease phenotype with that of an array of previously localized genetic markers. DNA from this region is then cloned and candidate genes are identified by study of their expression products. Once the mutant gene is conclusively identified, the role of the expression product of the normal allele (genetic variant) in cellular physiology is determined, and the molecular pathology resulting from each mutation can be examined.

The recent localization of the mutations for Huntington's disease [6, 7], ADPKD [8], and cystic fibrosis [9–12] has placed considerable emphasis on this type of approach. Successful application of this method has recently led to the isolation of sequences from the Duchenne muscular dystrophy (DMD) [13], chronic granulomatous disease [14], and retinoblastoma loci [15]. In the case of muscular dystrophy, investigators have isolated genomic and cDNA sequences for the DMD gene [13, 16–18], deduced the DMD protein sequence [17, 18], characterized DMD mutations and analyzed their phenotype/genotype relationship [18, 19], generated antibodies to a DMD-trpE fusion protein expressed in vitro [19], and used these to study the cellular distribution of the DMD gene expression product, dystrophin [20]. Although even in the case of DMD the ultimate goal of the reverse genetic approach (which is to explain the roles of the normal and mutant gene products in normal cell physiology and disease pathophysiology) is yet to be realized, the information gained from this approach has already had considerable impact on our understanding of DMD.

The localization of the ADPKD mutation to the short arm of chromosome 16 (16p) by Reeders et al. [8] was the first step in the reverse genetic approach to this disorder. This chapter will deal with this approach in detail and describe its application to ADPKD, reviewing current knowledge of the molecular genetics of this chapter, and describing current strategies being adopted in our laboratory.

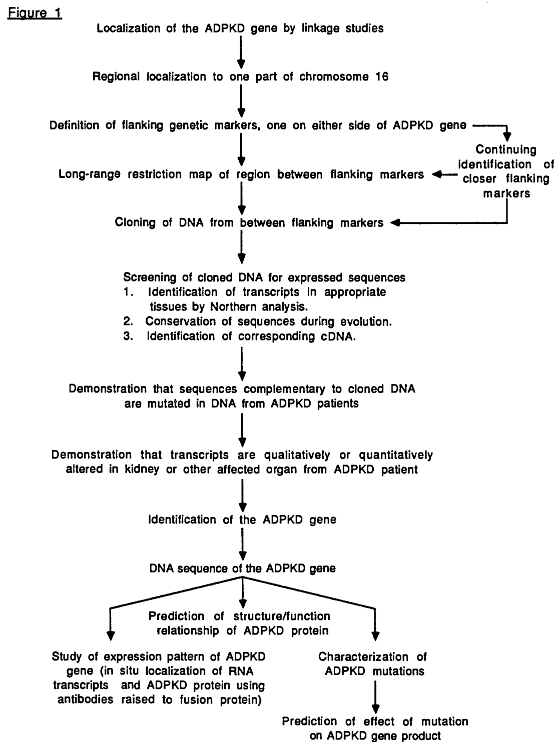


Figure 11-1. A schematic overview of the reverse genetic approach applied to ADPKD.

GENETIC LOCALIZATION

The genetic localization of a mutation is the process by which a mutant gene is assigned to a specific chromosome. In some disorders, prior knowledge of the disease suggests the candidacy of mutations in specific gene products as being causative. In such cases, the study of genomic DNA from affected patients using cloned sequences from the candidate gene (whose chromosomal assignment is already known) may readily identify and localize the mutations. An example of such analysis is the demonstration by Sykes et al. [21] that mutations in the $\alpha 1$ and $\alpha 2$ chains of type I collagen are each responsible for a proportion of cases of dominant osteogenesis imperfecta. In the case of ADPKD, basement membrane abnormalities had been postulated as being responsible for cyst formation in some models of pathogenesis, but initial studies ruled out a number of membrane components as candidate genes (Reeders, unpublished data).

Another clue to localization is sometimes provided by the association of

specific chromosomal abnormalities with a disorder. Thus, chromosome deletions were used to localize retinoblastoma to chromosome 13 [22, 23]. Although a number of cytological abnormalities have been detected in ADPKD patients, no clustering of specific abnormalities has been observed.

In the absence of candidate genes and cytological clues, localization of a mutation depends on the demonstration of genetic linkage with another genetically inherited characteristic, a genetic marker, whose localization is already known. It was this approach that localized the mutation for ADPKD to chromosome 16.

Linkage analysis

According to Mendel, genes (or genetic markers) on different chromosomes segregate independently during meiosis. However, closely spaced loci on the same chromosome are inherited together and are said to be linked (figure 11-2). In figure 11-2A, two unliked loci are considered. For each locus, an individual normally has two alleles, one on each partner of a chromosome pair. There are four different possible pairs of alleles from these loci, and these are randomly transmitted to offspring in a 1:1:1:1 ratio. When the loci are genetically linked as in figure 11-2B, allelic pairs are transmitted together to the offspring in a nonrandom fashion. However, linkage is seldom complete because, during meiosis, crossing over (recombination) of alleles between the maternal and paternal chromosomes occurs (Figure 11-2B). The rate of recombination is expressed as the recombination fraction, θ, and is determined by dividing the number of recombinations between two loci by the total number of meioses studied. The recombination fraction increases with distance between two loci and hence is taken as a measure of genetic distance.

Linkage is detected when the recombination fraction between two markers is less than 0.5. In figure 11-2A, random segregation of alleles from the unlinked loci results in $\theta = 0.5$, whereas in figure 11-2B, $\theta = 0.2$, which is consistent with the loci being genetically linked. However, since the alleles of unlinked loci are transmitted in a random fashion, it is possible that two loci appear to be linked if their alleles happen to cosegregate by chance. Figure 11-3 illustrates this point. Alleles of loci A and B cosegregate in 4 our of 5 offspring even though the gene loci are on different chromosomes. Therefore, a number of statistical methods have been devised for determining the probability that apparent linkage between two loci is by chance. The most widely used method of analysis is the likelihood method. In this method, the likelihood, $L(\theta)$, of the observed alleles is determined for a range of values of θ (from 0 to 0.5). θ is equal to 0.5 when loci are not linked, and $L(0.5)$ is the probability that the observed segregations occurred by chance. The odds ratio, or odds of linkage, is $L(\theta)/L(0.5)$. the log10 of this ratio is the LOD score and is the preferred statistic, since LOD scores may be summed for different data sets. The maximum value of the LOD score for a range of values of θ gives an estimate of

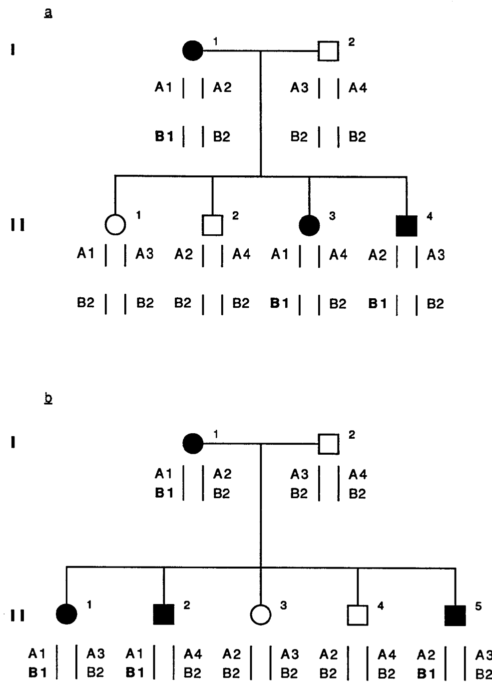


Figure 11-2. **A.** Locus B has a mutant allele (B1) that causes a disease (filled symbols). Homologous pairs of chromosomes are represented by vertical bars. Locus A has four different alleles and is a candidate genetic marker for the disease. However, in this example, A and B are on different chromosomes and hence no linkage is observed between them.
B. Loci A and B are closely spaced on the same chromosome. Allele B1 is the mutant allele, and it segregates with allele A1. Conversely, none of the normal individuals (B2) has allele A1. However, individual II-5 has the disease (B1) but allele A2, indicating that there has been a cross-over of alleles (recombination).

the true value of θ. A LOD score of three or more is considered firm evidence in support of linkage, corresponding to odds for linkage of at least 1,000:1.

Restriction fragment length polymorphism (RFLP)

Any locus with more than one allele is said to be polymorphic. The usefulness of any genetic marker depends on the number and frequency of its alleles in the population to be studied. In the example given in figure 11-4, the affected parent is homozygous for the marker allele A1 such that cosegregation of locus A with the disease cannot be tested. Consequently, one cannot assess genetic linkage between the marker and the disease (locus B), and such a family is said to be uninformative for that marker. Conversely, the affected parent in figure 11-2B is heterozygous at locus A, and cosegregation of the marker with the

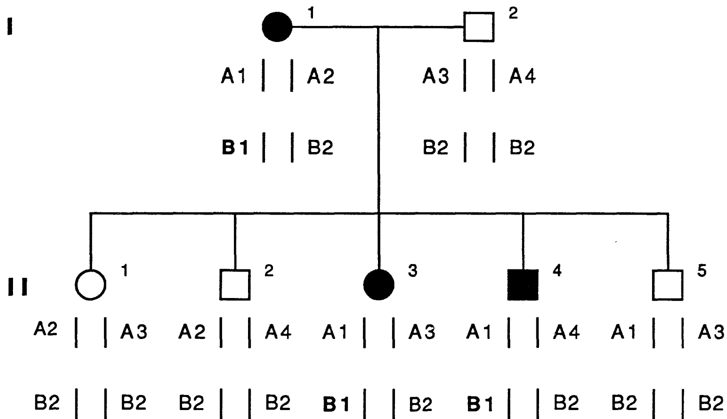


Figure 11-3. In this example, loci A and B are on different chromosomes, and B1 is the mutant allele responsible for the disease phenotype (filled symbols). Though the loci A and B are not linked, the marker A cosegregates by chance with B in this pedigree with a recombination fraction of 0.2.

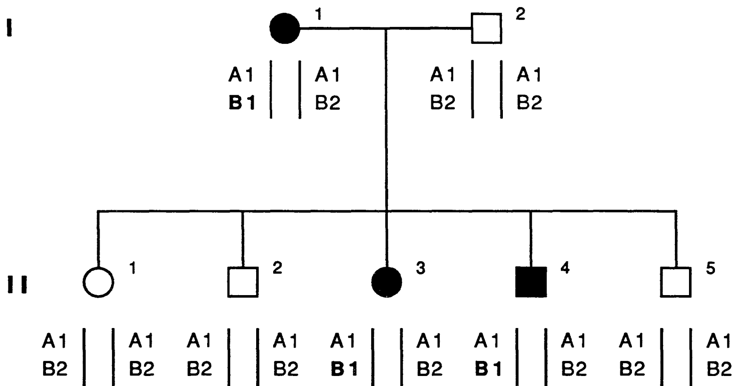


Figure 11-4. Locus B is a gene with a mutant allele B1 that causes a disease phenotype (filled symbols). Locus A is a candidate genetic marker. However, both parents are homozygous for allele A1, and the segregation of locus A with the disease phenotype cannot be tested.

disease is demonstrated. Therefore, it is clear that the greater the number of alleles at a particular locus, the greater the likelihood of heterozygosity at that locus, and the more likely it is that such a marker will be informative.

Prior to relatively recent developments in molecular biology, the only characteristics that were known to be polymorphic and hence useful as genetic

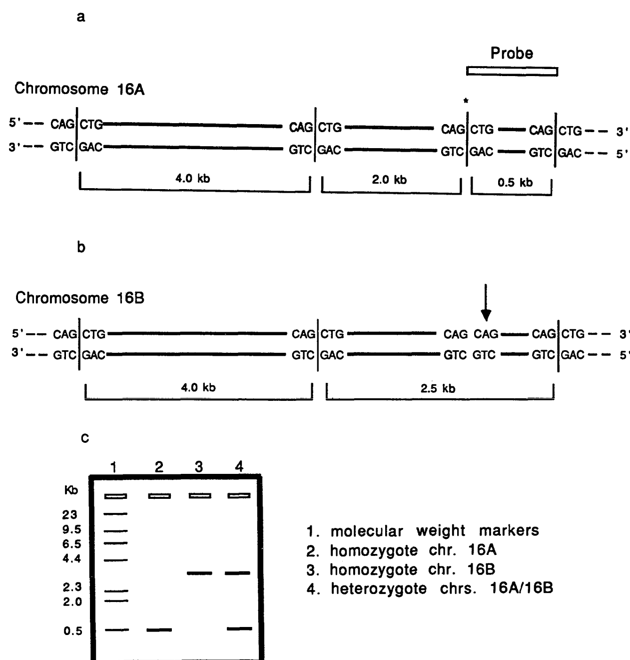


Figure 11-5. A, B. The partners of a chromosome pair are shown with sites for the enzyme Pvu II (vertical bars). The only difference between the two chromosomes is a point mutation (indicated by the arrow) within a Pvu II site that destroys that site (asterisk).
C. A schematic representation is shown of an autoradiograph of a Southern blot of Pvu-II digested DNA from three individuals, hybridized with radiolabeled probe. The probe is a sequence complementary to the 0.5-Kb Pvu II fragment. The probe recognizes different Pvu II fragments in each chromosome because of the absence of a Pvu II site on chromosome 16B—hence the term 'restriction fragment length polymorphism (RFLP).'

markers were other inherited diseases, functional differences (such as color blindness), blood groups, tissue antigens, and polymorphic proteins (such as phosphoglycolate phosphatase, or PGP). However, the discovery of restriction endonucleases has dramatically increased the potential for identifying genetic markers. This class of enzymes cleaves DNA in a sequence-specific manner (figure 11-5A). Therefore, DNA incubated with one of these enzymes is fractionated into a series of fragments the pattern of which is generally predictable for any given species. However, scattered throughout the genome are multiple sequence changes (often clinically silent) that may alter fragment sizes by creating new restriction sites or removing preexisting sites. The addition or removal of sites creates new lengths of DNA that can be recognized by hybridization of the complementary DNA sequence (figure 11-5B). DNA fragment size differences are called restriction fragment length

polymorphisms (RFLPs). They are Mendelian characteristics and are therefore useful genetic markers for pedigree analysis.

Strictly speaking, the RFLPs described above are dimorphic (two alleles), and this feature often limits their usefulness. Fortunately, there are regions of the genome in which the length of the DNA between two restriction sites varies considerably in a stable, inheritable fashion. These hypervariable regions (HVR) make particularly powerful genetic markers. It was such a marker that initially localized ADPKD to chromosome 16.

Genetic localization of ADPKD

After over 100 randomly picked loci had been analyzed, a hypervariable region that is 8 kilobase pairs (Kb) 3′ to the α-globin genes (3′HVR) and informative in over 90% of unrelated individuals was found to be closely linked to ADPKD. The 3′HVR was cloned by Nicholls et al. [24, 25] and consists of a 17-base-pair sequence repeated in a head-to-tail pattern (figure 11-6). The fragment produced by cleavage with the enzyme Pvu II, which cuts just outside this region, varies in length according to the number of copies of the tandem repeat within the 3′HVR. Variation in the size of the Pvu II fragment containing the repeats can be detected by gel electrophoresis, Southern blotting, and hybridization to a radiolabeled 3′HVR fragment that has been cloned into a plasmid. Figure 11-7 demonstrates that inheritance of this marker in an ADPKD family.

The initial studies were done on four British and five Dutch families; genetic linkage was observed in all families, with a maximum LOD score of 25.85, indicating that linkage was 10^{25} times more likely than nonlinkage in those families. These studies have been extended to include a total of 27 European families including some with an atypical clinical presentation; the recombination fraction, θ, between the ADPKD locus and 3′HVR at which the LOD score peaks (54.44) is approximately 0.08 for a male parent and 0.015 for a female parent [26, 27]. The difference between male and female recombination rates in this region is contrary to the pattern generally found within the genome, since female meiosis typically has a higher recombination rate. The mechanism that produces this sex-specific difference is not understood, but it does have practical significance in presymptomatic screening and genetic counseling.

A second ADPKD gene

An important question to be considered in any inherited disorder, and particularly in one with a variable clinical pattern such as ADPKD, is whether mutations in a single gene or gene cluster account for all forms of the disease. If this is the case, then linkage between the disease and a specific genetic marker will be observed in all families, whereas if mutations in more than one gene can lead to the disease, it will be linked to a specific genetic marker in some

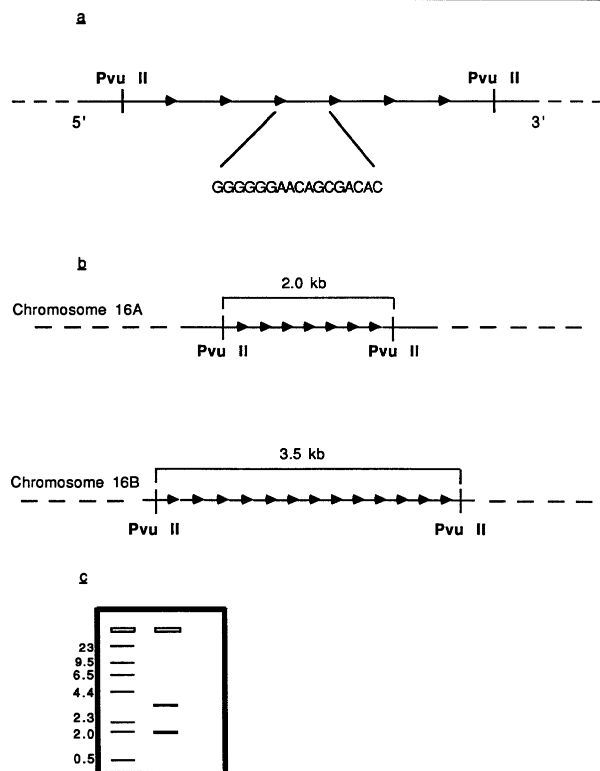


Figure 11-6. A. A schematic representation of the 3′ hypervariable region (3′HVR) from the α-globin region is shown. It is a tandemly repeated 17-bp sequence (sequence shown) with between 70 and 450 repeats within each 3′HVR [25].
B. The number of repeats in the 3′HVR is usually different in each partner of a homologous pair of chromosomes. The variability in the number of repeats results in a highly polymorphic genetic marker ideal for pedigree analysis.
C. The length of the 3′HVR that reflects the number of repeats on any individual chromosome is determined by Southern hybridization of DNA digested with Pvu II with a radiolabeled complementary probe. A schematic autoradiograph depicts two bands corresponding to the two alleles shown in figure 11-6B.

families but not in others. Heterogeneity in genetic linkage relationship is thus a good test for intergenic heterogeneity.

Initial studies failed to detect any evidence for genetic heterogeneity of linkage in ADPKD [26]. Ryynanen also demonstrated that ADPKD is linked to α-globin in a family in which ADPKD is not associated with renal failure [4]. These studies were confined to populations of Northern European origin. Recently, however, two families of Italian origin have been studied in which

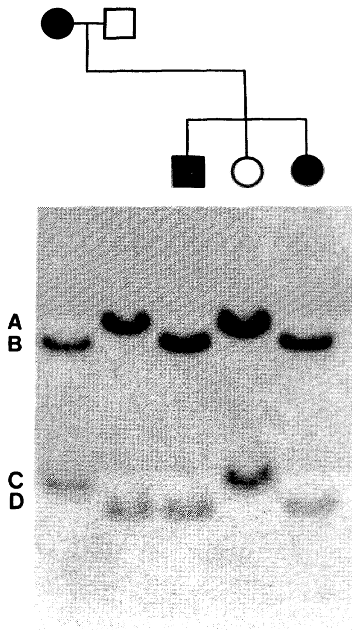


Figure 11-7. A sample PKD1 pedigree is shown with affected individuals indicated by filled symbols. Below is an autoradiograph demonstrating the 3'HVR alleles for each family member. Each track contains two hands, one from each chromosome. The variability in size is due to differences in the number of repeats within the 3'HVR on each chromosome. The alleles are labeled on the right. Allele B (from the mother) segregates with the disease.

no linkage between ADPKD and chromosome 16 markers was detected [28a]). The clinical presentation of the disease in these unlinked families could not be distinguished from the disease seen in previously studied linked families. The gene symbol PKD1 has been assigned to the form of the disease that is linked to α-globin; no symbol has, as yet, been assigned to the unlinked form or forms, since the genetic localization of the unlinked mutations has not been established. The relative frequencies of the two forms has not been accurately measured but, in the Northern European population at least, the unlinked form accounts for less than approximately 2% of all cases (S. Reeders, unpublished data).

Not all Italian families are unlinked. Del Senno [29] has reported linkage in several families from the Ferrara district, close to Bologna where one of the unlinked families originates [28]. Moreover, Soren Norby has studied a family from Copenhagen in which linkage between ADPKD and 3'HVR could not be demonstrated (S. Norby, personal communication). Currently,

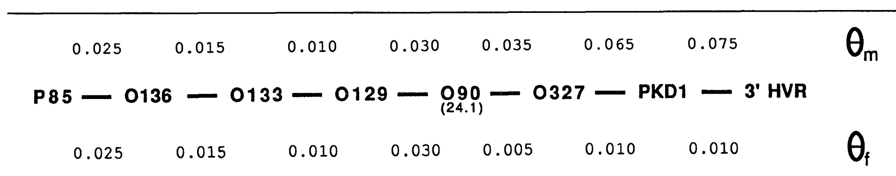


Figure 11-8. A linkage map of genetic markers about the PKD1 locus is shown. The order of the markers has been determined by multipoint linkage analysis. θm and θf are the recombination rates for male meioses and female meioses, respectively. Note that θm between 3′HVR and 0327 (the distal and proximal flanking markers closest to PKD1) is 0.14.

there is insufficient evidence to support or refute a geographical clustering of the unlinked forms of ADPKD.

Limits of localization

Prior to the localization of PKD1 to the short arm of chromosome 16 (16p), only α-globin [30] and phosphoglycolate phosphatase (PGP) [31] had been assigned to this region. PGP, a polymorphic protein, was then shown to be linked to PKD1 and α-globin [32, 33]. Although both 3'HVR and PGP localized the PKD1 gene locus to 16p, it was not possible to determine their orientation with respect to the telomere or centromere. Additionally, the region identified by these markers was predicted to be too large to be cloned by conventional strategies. Consequently, more precise mapping, directed at defining the limits of the position of the disease locus by identifying flanking markers, was pursued in collaboration with T. Keith and P. Harris using anonymous DNA sequences randomly picked from several chromosome-16-enriched libraries and screened for the ability to detect RFLPs. These markers have been ordered by tracing their inheritance through normal families destributed by the Centre d'Etude de Polymorphisme Humain, and in 28 PKD1 families. The results of these studies have been published in part [27, 34, 35] and a linkage map of this region is presented in figure 11-8. The recombination fraction between the closest flanking markers, 3′HVR and 0327, is approximately 0.14 in the male.

PHYSICAL LOCALIZATION

The flanking genetic markers, 0327 and 3'HVR, define the limits of the region that includes the PKD1 locus. Theoretically, it might be possible to clone all the intervening DNA between these loci and screen for candidate genes. Typically, cloning strategies involve *walking* along the chromosome by cloning sequential, overlapping fragments of DNA. To do this, one must have a library that contains genomic DNA sequences propagated as a manageable number of overlapping clones in a vector. Lambda bacteriophage (maximum insert capacity of approximately 20 Kb) and cosmids (which have components

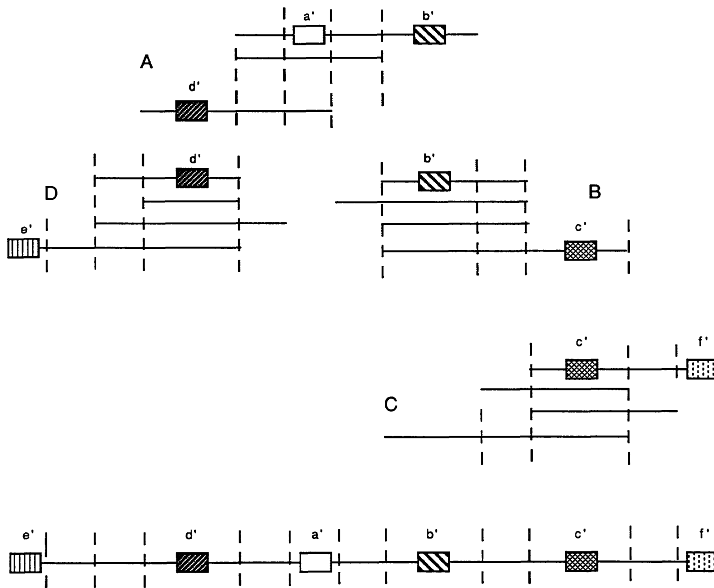


Figure 11-9. Chromosome walking: Sequence a′ is a genetic marker that is the starting point of the proposed walk. A radiolabeled probe is used to screen a cosmid or bacteriophage library, and a family of overlapping fragments is identified (A). b′ and d′ are sequences from the most proximal and distal ends of the 'A family' of fragments and are used to rescreen the library. Each identifies its own family of overlapping fragments, and the process is repeated. The walk proceeds in both directions until a landmark is crossed (a flanking marker, a translocation breakpoint, or a deletion endpoint), which orients the walk with respect to the centromere/telomere or the gene being cloned. In this diagram, broken lines represent the restriction sites that are the endpoints of each clone (i.e., the cloning site).

from both plasmids and bacteriophage and allow inserts up to 45 Kb) are most commonly used as vectors. The library is screened with a radiolabeled probe from the marker locus that is closest to the gene being sought. Those positive clones identified as containing the marker are then isolated, and fragments that lie at the extremities of these clones are radiolabeled and used as probes for further screening (figure 11-9). By this repetitive process, one gradually walks out in both directions until one crosses a landmark, such as another flanking marker, which thereby orients the walk and defines the limits of the region to be cloned. Using a cosmid library, each step of the walk averages only 20 Kb due to the overlapping of clones. The number of steps required will depend on the size of the region to be spanned. Walking along a region that stretches for several million base pairs (megabases) would require hundreds of steps and is impractical. Clearly, knowledge of the physical limits of the region to be spanned is extremely helpful in reducing the effort expended on walking.

Taking the human genome as a whole, 1% recombination (~1 centi-

Morgan, cM) corresponds to approximately 1000 Kb. There is reason to believe that 1cM corresponds to a much shorter segment in the PKD1 region. From the genetic map of existing probes, the genetic length of the terminal part of 16p (16p13) is at least 55cM in the male. If one assumes that 16p13 contains approximately 20 megabases (Mb), based on the DNA content of the whole chromosome, then 1cM on average corresponds to 400 Kb. Furthermore, of the 38 polymorphic sequences known to lie within 16p13, only 4 (3'HVR, 0327, 24.1, and c090) lie within 17cM of the distal end of the known genetic map (α-globin). Unless the physical distribution of these randomly picked probes is markedly nonrandom, it is likely that relatively high recombination occurs per unit physical distance in the region of the PKD1 locus. Such recombination hotspots have been reported for other parts of the human genome. The regional variability and sex-specific differences of recombination rates make estimating the physical size of a region based on recombination rates very inaccurate. A more precise map is necessary to determine the size of the PKD1 region if the map is to guide cloning strategies.

Cytogenetic mapping

Chromosomal abnormalities can be very useful in physical mapping strategies. These naturally occurring deletions and translocations can be used to define the physical limits of the region between flanking markers, to help orient markers with respect to the centromere or telomere, to help order probes with respect to each other, and even to guide the selection of candidate genes. Abnormal chromosomes can be especially useful in rapidly localizing new markers that may be generated by the methods discussed below.

Although no chromosomal abnormalities have yet been associated with ADPKD, a number of translocations, as well as several submicroscopic deletions from patients with hemoglobin H (a form of α-thalassemia in which 3 out of 4 α-genes are either deleted or aberrant) and mental retardation, have been identified [36]. Several mouse–human hybrid cell lines containing an abnormal chromsome 16 as the only human component have subsequently been produced in the laboratories of D.R. Higgs (Oxford), M.H. Breuning (Leiden), and D.F. Callen (Adelaide), as well as in our laboratury by N.J. Barton. In the case of hybrids, the localization of markers was determined by hybridizing probes to Southern blots containing digested hybrid DNA. An unbalanced translocation in which the terminal part of 16p was deleted was studied by Breuning et al. [37] and was used to demonstrate that α-globin was very close to the telomere and distal to the PGP locus. Each of the genetic markers about the PKD1 locus has subsequently been mapped with respect to several chromosomal breakpoints, and the data are summarized in figure 11-10. The physical localization of these marker loci confirms the data derived from linkage analysis and assists in orienting the PKD1 linkage group with respect to the chromosome, placing the α-globin cluster (with its 3′HVR marker) closest to the telomere.

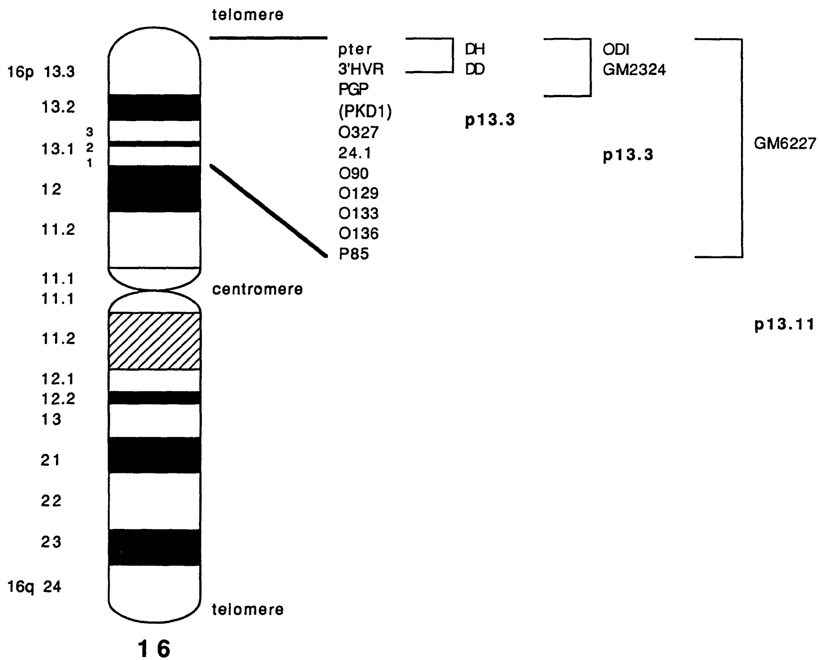


Figure 11-10. On the left is a schematic representation of a giemsa-stained metaphase chromosome 16. Immediately to the right of chromosome 16, a linkage map of the p13-pter region is presented with the most telomeric sequence at the top. Further right, the position of the breakpints of a number of translocations and deletions are shown with respect to the map of polymorphic probes. The position of the PKD1 locus with respect to the deletion endpoints and translocation breakpoints is not known except for GM 6227. Hybrids and cell lines were studied in collaboration with D.R. Higgs, C. Hatton, P. Harris, and N. Barton (Oxford) and M. Breuning (Leiden).

Restriction mapping

Since chromosomal abnormalities need to be at least one megabase in size to be detected by cytogenetic study, the resolution of cytogenetic mapping is limited and inadequate for guiding cloning strategies. Consequently, a restriction map (a physical map of restriction sites for a variety of enzymes) is a necessary prerequisite for cloning. It converts the genetic distance expressed in recombination rates into base-pair units, allowing accurate prediction of the size of the region to be cloned. Such a map can also be used to localize cloned sequences.

Restriction mapping typically requires digestion of DNA by restriction endonucleases and size separation by continuous single-field electrophoresis. However, this technique cannot resolve fragments greater than 40 Kb. Since the genetic distance between the flanking markers is much larger, it could take

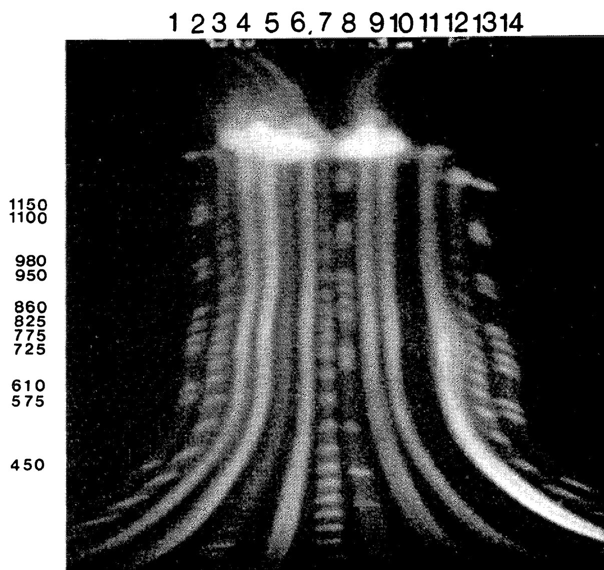


Figure 11-11. Pulsed field gel electrophoresis (PFG) of human DNA. The samples were digested with an infrequent cutter (see table 11-1) and separated in an inhomogeneous field apparatus for 108 hours at 225 volts with the electric field switched through 90 degrees every three minutes. Yeast chromosomes (Saccharomyces cerevisiae, strain CY14) in lanes 1, 8, and 14 and bacteriophage concatemers (48 Kb monomers) in lanes 2, 7, and 13 serve as markers. The sizes to the left are expressed in kilobases. Note the track distortion; this is a consequence of the inhomogeneous electric field. Lanes 3–6, 9–11: seven different samples of genomic DNA digested with Nru 1. Lane 12: genomic DNA digested with Cla I.

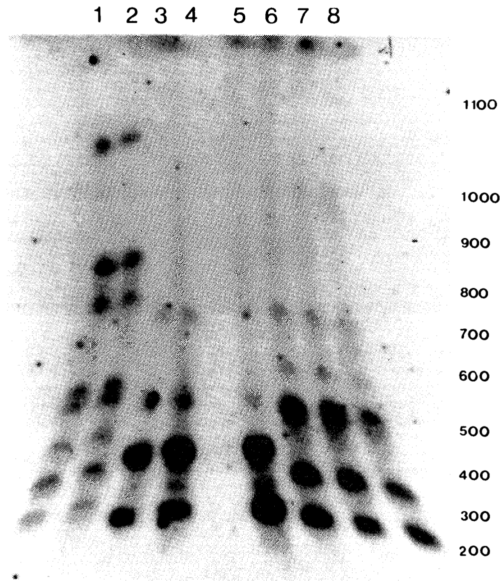
hundreds of markers to complete a map linking them. Fortunately, a number of strategies have been developed to reduce the effort required to produce maps stretching across megabase regions of the genome. One recently developed technique, pulsed field gel electrophoresis (PFGE), bridges this gap in mapping between cytogenetic/genetic distances and the limits of classical single-field electrophoresis. DNA molecules greater than 25 Kb, which ordinarily migrate independent of size in single-field electrophoresis, are able to migrate in a size-dependent fashion when the direction of the electric field is periodically switched. The prototype, developed by Schwartz and Cantor [38], employed alternating orthogonal fields (figure 11-11). A more recent modification by Chu et al. [39] produces contour-clamped homogeneous electric fields (CHEF) that are switched through 120°. This technique produces less distorted tracks, facilitating their comparison and allowing more accurate size determinations. Both the orthogonal field and CHEF devices are able to resolve DNA in the range 20 Kb to 9000 Kb [38, 40], the limits of resolution being determined by the pulse interval. Longer pulse times are necessary to

allow larger molecules sufficient time to change their conformation in response to the change in direction of the voltage gradient. Once the run is completed, the gel is treated with ultraviolet light to break up the larger fragments, thereby improving their transfer. DNA is then transferred to reusable nylon membranes by standard Southern blotting protocols and hybridized with single copy probes (figure 11-12A,B). Lambda bacteriophage concatemers (1-mer to 25-mer, 48 Kb to 1200 Kb) and intact chromosomes from various yeast strains (*Saccharomyces cerevisiae*—range 230 Kb to 1600 Kb, and *Schizosaccharomyces pombe*—range 3 Mb to 9 Mb) are run as size markers.

Besides guiding cloning strategies, long-range restriction mapping using the technique of PFGE may identify areas that are especially gene-rich. This additional benefit results from a fortunate coincidence: many of the enzymes used to generate very large fragments have restriction sites clustered about the 5′ ends of genes. The low frequency of cleavage for these enzymes is based on their 6–8 base-pair specificities and the presence of a CpG dinucleotide in their recognition sequence (table 11-1). CpG dinucleotides are underrepresented in the genome. Additionally, the cytosine base is often methylated in vivo, and this methylation blocks cleavage by most of the enzymes used (Cla I, Not I, Nru I, Mlu I). However, there are regions of 1–2 Kb in length (Hpa II Tiny Fragment Islands) in which there is a relative increase in the frequency of the CpG dinucleotide, usually in the unmethylated state [41]. These islands are important because they frequently mark the 5′ end of genes, having been found to occur at the ends of nearly all of the sequenced housekeeping genes that are transcribed by RNA Polymerase II [41, 42]. HTF islands have also been found at the 5′ ends of a number of tissue-specific genes (e.g., α-globin). The infrequent cutting enzymes used to generate large fragments for PFGE mapping have recognition sites concentrated at HTF islands. Therefore, HTF islands may frequently be localized as a cluster of infrequent cutting enzyme sites [42]. In this way, the PFG map of the PKD1 region may help identify candidate genes. These enzymes and the technique of PFGE have been successfully used in constructing long-range maps about the human major histocompatibility and DMD loci [43–47].

Long-range mapping about the PKD1 locus

Preliminary results are summarized in figure 11-13. Short-range restriction mapping about the 3′ and 5′ hypervariable regions of the α-globin complex was initiated by Fischel-Ghodsian et al. [48]. Our laboratory has extended this map by studying the use of additional enzymes that prove to have less frequent restriction sites in this region (Cla I, Nru I) and by developing controlled partial digest mapping. Each of the markers from this region has been similarly analyzed. Although the data are incomplete, several observations are noteworthy:



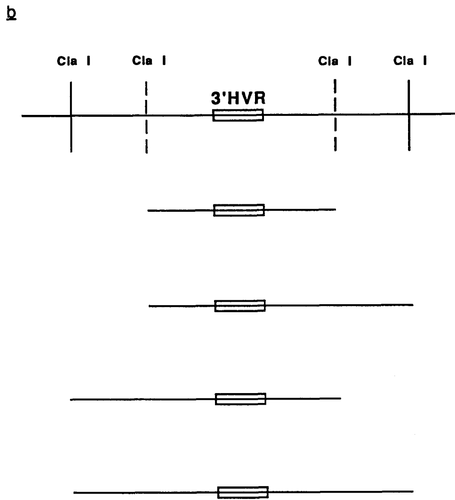


Figure 11-12. A. Southern hybridization of PFG-separated DNA. The autoradiograph shows the hybridization of 3′HVR to human DNA digested with Cla I (lanes 1,2), Nru I (lanes 3–5), and SnaB I (lanes 6–8). Numbers to the right are size markers (in kilobases). The large number of bands recognized by 3′HVR in each track is due to partial digestion.
B. This is a schematic representation of partial digestion as seen in figure 11-12a. The Cla I sites identified by a hatched line do not cut to completion, whereas those identified by a solid line are always cleaved. Hence, hybridization with 3′HVR will detect a ladder of overlapping fragments.

Table 11-1. Eleven infrequent-cutter enzymes and their recognition sequences

Enzyme	Sequence
BssH II	GCGCGC
Cla I	ATCGAT
Eag I	CGGCCG
Mlu	ACGCGT
Not I	GCGGCCGC
Nru I	TCGCGA
Pvu I	CGATG
Sal I	GTCGAC
Sfi I	GGCCNNNNNGGCC
SnaB I	TACGTA
Sst II	CCGCGG

1. Despite identifying fragments of up to 1.2 Mb in size with 3'HVR and 2–3 Mb with 24.1 (the distal and proximal flanking markers, respectively), physical linkage has not been demonstrated. Therefore, the distance between the flanking markers is at least several megabases and too great to be conveniently covered by cosmid overlap walking.

2. There is considerable regional variation in the fragment sizes produced by these infrequently cutting enzymes. Digestion with Not I, an enzyme with recognition sequence GCGGCCGC, typically produces fragments in the megabase range. Indeed, around the proximal flanking markers 24.1, LOM2B, and 090a, a very large Not I fragment of approximately 2–3Mb is detected (G. Germino, unpublished results; A.M. Frischauf, personal communication). However, 3'HVR recognizes a Not I fragment of only 200 Kb, and cosmid mapping about the α-globin complex has identified two Not I sites within 5 Kb [48]. Smith et al. noted a similar phenomenon in their study of a chromosome-3-specific cosmid library [49]. Both in their cosmid library and in our pulsed field data of the 16p13.3 region, a clustering of infrequent restriction sites has been observed. Sequence data of approximately 24 Kb about the α-globin complex [48] reveals this area to be more GC-rich (60.6% G+C) than most other regions of the genome, and CpG suppression has been reported to be reduced in GC-rich areas [50]. Zerial et al. [51] had previously shown that the α-globin complex is contained within a GC-rich fraction of relatively homogeneous GC content that they called an isochore. Fischel-Ghodsian et al. [48] suggested that this isochore extended for at least 300 Kb about the α-globin complex. Preliminary data from our lab (unpublished) suggests that this isochore extends through the PKD1 locus but ends before reaching the proximal flanking markers. This clustering of infrequent-cutting enzyme sites is important for two reasons:

a) It suggests that this region may have many HTF islands and consequently be gene-rich.

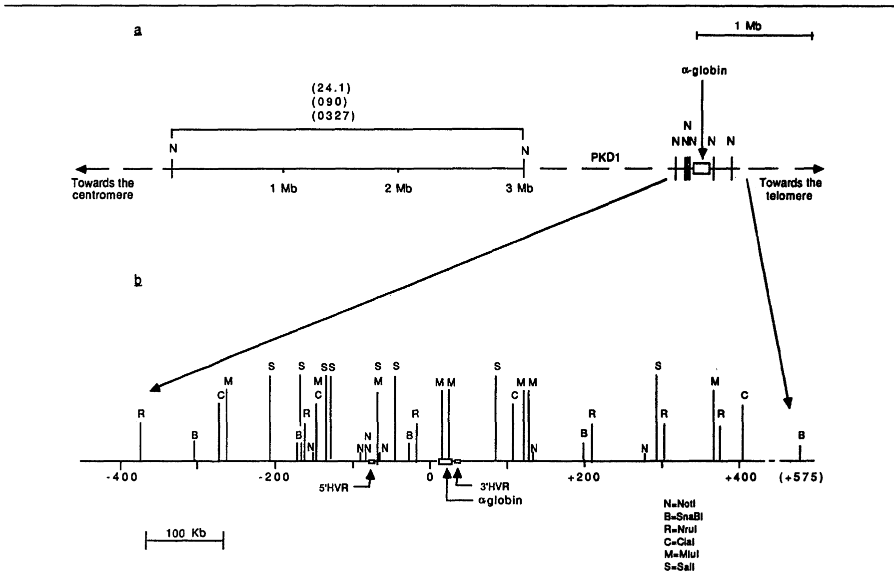


Figure 11-13. **A.** Preliminary Not I restriction may of 16p13. The orientation of 24.1, 0327 and 090 with respect to each other and the ends of the Not I fragment is not known. The distance between these markers and the α-globin complex has not been determined.
B. Long-range restriction map of the α-globin complex. The orientation of the 3′HVR and 5′HVR loci with respect to the telomere is not known. The location of restriction sites for some of the enzymes has been approximated. (Much of this work has been done by N. Fischel-Ghodsian et al. [48].)

b) It may be time-consuming to link up marker loci by PFGE because the fragments are so small that a large number of probes may be required to identify contiguous fragments.

Generating new markers

Although our long-range maps about the flanking markers 24.1 and 3′HVR are incomplete, it is likely that currently available probes will not identify a contiguous set of fragments spanning the region between the closest proximal and distal markers, and further probes may be required. One could generate new probes by screening chromosome-16-enriched libraries for markers that localize to the 16p13 region, but this is a very tedious process. Ideally, one would prefer to be able to jump out a large distance from a known, circumscribed region into another, flanking region, possibly crossing restriction sites that are always completely digested and have thereby thwarted attempts at linking up flanking regions. One useful approach is to screen with hopping probes. These were first described by Collins and Weissman [52] and contain

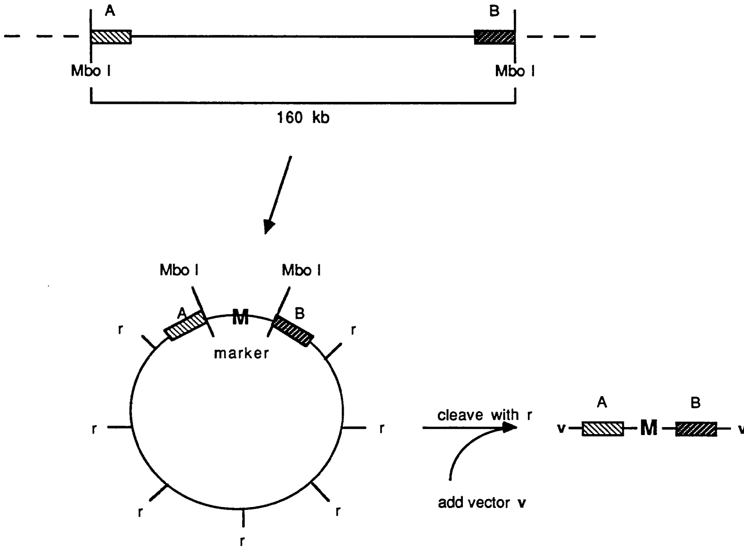


Figure 11-14. Hopping probes: DNA is cleaved into large fragments either by digestion with an infrequent cutter (table 11-1) or by partial digestion with a more frequent cutter (Mbo 1 in this example). The fragments are circularized in the presence of an excess of a selectable marker (**M**), which is incorporated into the circles at the junction of A and B. The circles are digested with a second enzyme with sites illustrated in the diagram by slashes (r). The fragments are then ligated into a vector **v** (cut with r) and grown on medium selecting for those clones with **M**. Those clones that grow contain only the ends (A,B) of the 160 Kb fragment [52].

just the ends of a large fragment (figure 11-14). A library of these probes is made by circularizing large DNA fragments with selectable markers at the point of ligation. These are then digested with a second enzyme, which cleaves out most of the genomic DNA, and then cloned in selective media, which rescues clones that contain these junction fragments. If partial digests are used to prepare the DNA for hopping, it is possible to use a probe from the distal end of the first hop to screen the junction library for a second hop, and in this way, to proceed in a directional fashion along the chromosome. The experience of several laboratories (using several hopping libraries) confirms our finding that the probability of completing a single hop is approximately 60% for an average hop of about 180 Kb, so that the chance of covering two hops (360 Kb) by this technique is only around 36% (K.E. Davies, D.R. Higgs, personal communication). Although it is likely that this strategy will generate new markers, sequential hopping from one flanking marker to the other may not be possible.

Preparative gels have been successfully used as another way of generating new markers [53]. Based on the pulsed field map, an enzyme is chosen that predictably produces large fragments containing one of the genetic markers.

DNA from a chromosome-16-only mouse/human hybrid is digested with this enzyme and electrophoresed in a CHEF apparatus, and the appropriately sized fragment is sliced from the gel and electroeluted. It is then cloned into bacteriophage, and screened for human clones. Although this minilibrary will include mouse sequences as well as similarly sized fragments from other regions of chromosome 16, there should be a 10–100-fold reduction in complexity, with a relative enrichment for the region of interest. Human clones (identified by screening with a human-specific sequence) can be rapidly mapped by hybridization to the panel of hybrids described earlier (figure 11-10).

It is anticipated that a combination of the above strategies will ultimately identify enough markers to allow construction of a map that physically links the proximal and distal flanking markers for PKD1.

CLONING THE REGION ABOUT THE PKD1 GENE

The ultimate goal of the numerous mapping strategies is the isolation and cloning of the PKD1 gene. The aim is to clone the region defined by the flanking markers and subsequently screen for candidate genes. Regions that are less than 200 Kb can be readily isolated by chromosome walking. However, walking along a region that stretches for several megabases would require hundreds of steps and is impractical. Therefore, if the pulsed field map of the PKD1 region suggests that the area to be cloned between the closest flanking markers is very large, an alternative, complementary strategy requiring preparative gels will be employed. Fragments identified by pulsed field mapping that contain the closest proximal and distal flanking markers (and hence the PKD1 gene) will be eluted and subcloned into bacteriophage, thereby producing a regional library of the segment of DNA about the PKD1 locus. Clones identified as coming from within the region of interest will be screened for candidate genes as described in the next section.

IDENTIFICATION OF THE PKD1 GENE

The identification of the mutated gene from within the mapped and cloned region is clearly a critical step in the reverse genetic process. This may be especially challenging when surveying a region such as 16p13, which is likely to be gene-rich. Determining that a sequence from this region is expressed will not, in itself, prove that the sequence is from the PKD1 gene. Therefore, a number of complementary techniques must be used to identify candidate PKD1 sequences. None of these techniques, used in isolation, will reliably pick out all candidates. A theoretical discussion of the use of these methods follows.

Gene expression

The hallmark of a candidate sequence is that it is expressed. A candidate sequence must fulfill this criterion before major effort is expended on detailed

characterization. Since one does not know the protein product of the normal or mutated gene, other methods must be used to screen for expression. Northern analysis is the standard way of detecting transcripts of candidate sequences. In this technique, purified RNA is extracted from tissue and electrophoresed through agarose. The RNA is then transferred to a nylon membrane by Northern blotting. The membranes are subsequently hybridized to radiolabeled candidate DNA sequences, and transcripts are identified as bands by autoradiography. Because it is not known in which tissues and at which stages of development the PKD1 gene is expressed, a wide variety of tissues at various stages of development will be used. It is still possible, however, that hybridization of candidate sequences to mRNA will not be detected because the transcript is only present in very small amounts. Therefore, Northern analysis may be supplemented by screening cDNA libraries prepared from kidneys at various stages of development. cDNA libraries represent DNA copies of the mRNA population from a particular tissue or cell type. If representative cDNA libraries are constructed, it is possible to isolate cDNA clones of rare mRNAs that might be undetected by Northern analysis.

Sequence conservation

It has been observed by evolutionary biologists that the sequences of many genes are highly homologous between species, and such genes are said to be conserved. It is thought that those sequences that are essential to function are less likely to diverge over time, since mutations in these sequences are likely to lead to impaired function. Therefore those sequences that are highly conserved have a higher likelihood of being genes. This strategy has been used successfully by Monaco et al. [16] to isolate expressed sequences from the DMD gene. Although conservation is not always indicative of expression, and although not all expressed sequences are conserved, this technique does in many cases overcome the problem of identifying sequences that are either expressed at very low levels or are only expressed in specific cell types at specific stages of development. Conservation is assessed by hybridizing candidate sequences to genomic DNA from a panel of species,—a process known as *zoo blotting*.

HTF islands

The pulsed field map about the PKD1 locus identifies HTF islands as a clustering of infrequent cutter sites. Cloned DNA may also be screened for islands as a way of identifying candidate genes. Although there is no way of currently predicting whether or not the PKD1 gene is flanked by HTF islands, this strategy has been used successfully by Rappold et al. [54] to identify a testis-specific gene from the mouse t-compex, and by Esitvill et al. [55] to find candidate genes for cystic fibrosis.

Search for deletions and rearrangements

To date, no deletions or other rearrangements have been detected in PKD1 patients either at the cytogenetic or molecular levels. Moreover, it is not

possible to predict a priori whether naturally occuring deletions or other rearrangements of a size sufficient to be detected by restriction mapping do in fact occur in PKD1. However, the high spontaneous mutation rate and the marked variation in the phenotype suggest the possibility that at least some PKD1 mutations are deletions. The identification of deleted or rearranged regions in affected patients strongly points to the site of the ADPKD gene.

Summary of criteria for identification of PKD1 gene

In the initial stages, evidence suggesting that a particular candidate sequence is the PKD1 gene will of necessity be circumstantial. During this phase, it will be of primary importance to find eough evidence incriminating a given candidate sequence so that effort is not wasted on irrelevant genes. It is likely that evidence that is sufficient to prove involvement will only accrue gradually during the course of study of the organization of the gene and its mutations. However, there are clearly a number of minimum criteria:

1. Expression of the candidate gene must be shown to be abnormal in PKD1 patients;
2. Mutations of the gene itself or of a regulatory sequence must be found in patients; and
3. Tissue-specificity of expression must be appropriate.

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12. EPIDEMIOLOGY OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: IMPLICATIONS FOR GENETIC COUNSELING

PATRICIA A. GABOW

Among all hereditary renal diseases, autosomal dominant polycystic kidney disease (ADPKD) stands as the most common. In fact, even outside of the confines of renal disease, it is one of the most prevalent genetic maladies, being five to ten times as common as cystic fibrosis or sickle-cell disease [1, 2]. The frequency of ADPKD is between 1 in 200 and 1 in 1000, and thus, it is estimated that between 250,000 to 1,000,000 people in the United States are afflicted with the disorder [3–5]. Although the disorder is not confined to any specific ethnic or racial group and appears to be world-wide in distribution, there is a lower reported frequency in blacks [6–8]. The disorder is of importance to the clinician not only because of its frequent occurrence, but also because of its myriad manifestations. Clinically, ADPKD is characterized by the development of renal cysts and extrarenal manifestations including gastrointestinal, cardiovascular and musculoskeletal abnormalities (Table 12-1).

Physicians caring for and counseling patients with ADPKD and their families need answers to five questions:

1. What clinical information is required to establish the diagnosis?
2. What are the clinical manifestations of the disorder?
3. What is the natural history of ADPKD?
4. What is appropriate management for ADPKD?
5. Which members of ADPKD families should be evaluated for the disorder and with what method?

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Table 12-1. Organ system involvement in ADPKD

Genitourinary system
Renal cysts
Gastrointestinal tract
Hepatic cysts
Pancreatic cysts (rare)
Colonic diverticulae
Cardiovascular system
Cardiac valvular abnormalities
Berry aneurysm
Thoracic aortic aneurysm
Musculoskeletal
Inguinal hernias

The answers to these questions are somewhat different in children and adults. Therefore, these two age groups will be discussed separately.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE IN CHILDREN

Prior to the advent of genetic linkage and other recent gene identification techniques, definition of the inheritance patterns of a hereditary disease was based on the application of the principles of Mendelian genetics to clinical observations from affected families. In this manner, two types of polycystic renal disease emerged: autosomal recessive and autosomal dominant disease. Autosomal recessive polycystic kidney disease (ARPKD) generally appeared in infants and young children, while autosomal dominant polycystic kidney disease (ADPKD) generally produced clinical manifestations in the adult. From these generalities regarding clinical onset of disease, the genetic nomenclature was translated into the clinical nomenclature of infantile polycystic kidney disease and adult polycystic kidney disease. Thus, children diagnosed as having renal cystic disease were presumed to have autosomal recessive disease. Infantile or childhood presentation of the autosomal dominant was considered a curiosity to be described in isolated reports [9–25]. Inadequate family histories, failure to formally evaluate parents of affected children for renal cystic disease, and lack of large prospective studies to screen infants and children at 50% risk for ADPKD with sensitive techniques, all contributed to the failure to diagnose ADPKD in children. In one large study of presumed ARPKD, both parents were formally evaluated for renal cystic disease in one of 14 instances [26]. In another recent study of infantile renal cystic disease, screening of parents was not a necessary criteria for diagnosing ARPKD [27]. This in fact is a critical requirement for the diagnosis of ARPKD, since ADPKD often does not produce clinical symptoms until after the onset of childbearing. Thus, an affected parent might be unaware that he or she has

ADPKD until an offspring is diagnosed with renal cystic disease and the parent and offspring undergo renal imaging. In fact, in one study only 38% of the affected parents who had infants with ADPKD were aware of their status prior to conception of an affected child [28]. Despite the past confusion in categorizing childhood renal cystic disease, it is now clear that ADPKD may commonly present early in life [27–30] and thus this disorder must be considered a likely diagnostic possibility for children with a family history of polycystic kidney disease. In a recent series, 12% of children with cystic disease who survived the first month of life and were diagnosed in the first year of life had ADPKD [27]. In addition, another study revealed that in 12 of 25 families with infants who survived the neonatal period and had renal cystic disease, the infants had ADPKD [30].

The discussion of ADPKD in childhood should primarily focus on the five questions raised above.

What clinical information is required to establish the diagnosis?

The clinical diagnosis of ADPKD in childhood relies primarily on renal manifestations, construction of a reliable family pedigree, and the absence of hepatic fibrosis or portal hypertension. The focus on the phenotypic manifestation of renal cysts gives rise to several obvious questions. For example, "Are bilateral renal cysts a prerequisite for the diagnosis?" "Is a single cyst in each kidney an acceptable diagnostic criteria?" "Are normal-sized kidneys compatible with ADPKD?" There are no definite answers to these queries, but reasonable preliminary answers are found in the study by Sedman et al. [29]. In that study, 154 children from 83 ADPKD families were studied, three with excretory urography and 151 with abdominal ultrasonography. Children were classified by arbitrarily defined criteria that would be conservative in classifying simple cysts as ADPKD. This was accomplished by creating three diagnostic categories. Definite ADPKD was diagnosed if bilateral renal cysts totaling five or more were identified. Children with fewer or unilateral cysts were considered suspicious, and children with no detectable renal cysts were considered unaffected. The follow-up on the children sheds some light on the interpretation of cyst number and bilaterality of cysts in children. Seventy-one percent of 14 suspicious children who were seen a mean of 6.8 years after initial classification progressed to definite ADPKD. Further longitudinal studies will be needed to absolutely clarify the interpretation of renal cysts in children from ADPKD families. However, given these initial data and the infrequent finding of renal cysts in randomly selected children [31], any child in an ADPKD family with any renal cysts should be considered likely to have ADPKD. The requirements of bilateral cysts and five or more cysts appear to be too conservative a diagnostic criteria. Children with suspicious renal findings should be reexamined with imaging techniques at two to three year intervals if the diagnosis is to be made by that modality. Because they are likely to have the ADPKD gene,

Table 12-2. Diagnostic criteria for ADPKD in childhood

1. The presence of any renal cysts in a child at 50% risk for ADPKD
2. A documented family history compatible with autosomal dominant inheritance including renal imaging studies of parents
3. Clinical and ultrasonographic absence of hepatic abnormalities. In some instances, liver biopsy may be necessary.

hypertension and urinary tract infections should be viewed in the same manner as in ADPKD children (see below). Children with cysts should be examined at least yearly to determine blood pressure.

In contrast to the progression in children classified as suspicious, children with a normal renal sonogram at 10 to 12 years of age appear much less likely to develop features suggesting ADPKD [29]. Only 7 of 37 (19%) of unaffected children exhibited some progression during 11.2 years of follow-up [29]. Four of the seven progressed to definite ADPKD, but not until their early thirties. Thus, it appears that children with a normal ultrasonography should not be rescreened for at least four to five years in the absence of altered clinical status.

These groups of affected, suspicious, and unaffected children were all young, with mean ages of 10 to 12 years; thus absence of renal cysts in the unaffected group was not simply a reflection of age selection or lack of sensitivity of ultrasonography in the young. In fact, the utility of ultrasonography is demonstrated by the identification of the 23 definite ADPKD children among the study population [29]. Ultrasonography can assess renal size as well as renal cyst number. Although the kidneys can be enormous in childhood ADPKD presenting as abdominal masses, the increase in size need not be impressive, and in fact children can have normal-sized kidneys. Detectable renal cysts, coupled with family history and clinical evaluation can be utilized to establish the diagnosis (tables 12-2 and 12-3). However, even in the face of detectable renal cysts the diagnosis of ADPKD can still be elusive in childhood, as demonstrated by cases 2 and 3 in table 12-3. These cases illustrate the utility of genetic linkage analysis in these diagnostic dilemmas.

Case 1 clearly has ADPKD with positive family history and bilateral renal cysts and no evidence of hepatic dysfunction. From the data given above regarding renal cysts in a child at 50% risk, the child in case 2 has a 70% likelihood of developing ADPKD in about seven years. In addition, this child displays a common extrarenal manifestation of ADPKD, namely mitral valve prolapse. Although there is no longitudinal data to attach a relative risk figure to this cardiac abnormality, it increases the likelihood of ADPKD, and this patient should be considered by the clinician as affected. Because some finite possibility exists that the patient does not have ADPKD, it may be wise to withhold attaching a label of ADPKD, particularly in light of future insurability. The patient in case 3 is much less clear. This child was initially diag-

Table 12-3. Patterns and ADPKD in childhood

	Case 1	Case 2	Case 3
Patient's age (years)	7	8	6
Mother's age (years)	36	33	31
Mother's status	Affected—ADPKD Bilateral renal cysts Hepatic cysts Mitral valve prolapse	Affected—ADPKD Bilateral renal cysts Hepatics cysts Mitral valve prolapse	Normal-sized kidneys Single renal cyst
Father's status	Unaffected	Unaffected	Unaffected
Maternal grand-parents' status	Affected grandfather	Affected grandfather	Grandfather, age 56: single renal cyst
Paternal grand-parents' status	Unaffected	Unaffected	Unknown
Siblings' status	1 of 2 siblings <5 cysts 1 sibling: spontaneous abortion 1 sibling: therapeutic abortion for ADPKD	2 of 2 siblings have renal cysts <5 in each kidney	12-year-old brother: normal 10-year-old brother: increased kidney size, hyperechogenic 8-year-old brother: normal 3-year-old sister: increased kidney size, liver hyper-echogenic
Patient ultra-sonogram renal	6–15 cysts, both kidneys	Single cyst in each kidney	Increased kidney size and echogenecity
Patient renal function	Normal	Normal	Normal
Patient hepatic function	Normal	Normal	Normal
Extrarenal ab-normalities	None	Mitral valve prolapse	2 liver cysts

nosed as ARPKD largely based on age of onset. The presence of renal abnormalities in other family members, particularly the mother and maternal grandfather, somewhat complicates the diagnosis. Unlike the situation with children, isolated renal cysts have a high frequency in the random adult population. By 50 years of age about 50% of the subjects may have a renal cyst [32]. Thus, the maternal grandfather may in fact be normal. However, the presence of a renal cyst in the 31-year-old mother is less common, although not rare. Moreover, the occurrence of renal cysts or increased echogenicity in

three of five siblings may be somewhat more likely in autosomal dominant than in autosomal recessive disease.

Does the ultrasonography image or the excretory urographic appearance aid in distinguishing ARPKD from ADPKD? Classically, ARPKD in ultrasonography reveals enlarged kidneys with increased echogenicity; clearly defined cysts are rarely seen. Moreover, the sensitivity and specificity of ultrasonography appearance remain to be determined, since ultrasonographic appearance similar to ARPKD has been observed in young infants with ADPKD [28]. Similar dilemmas exist with excretory urography. In a recent study, only one of six infants was correctly diagnosed as ADPKD by excretory urography [27]. In this patient the ultrasonography was interpreted as compatible with autosomal recessive disease.

The interpretation of the extra-renal manifestations is also difficult. In ADPKD the presence of hepatic cysts prior to 20 years of age is very unusual. The typical hepatic abnormality in ARPKD is hepatic fibrosis, not hepatic cysts. This highlights the potential value of liver biopsy in children whose clinical diagnosis is confusing. If hepatic fibrosis were present in case 3, the weight of the clinical data would favor the dignosis of ARPKD.

Is there a role for genetic linkage analysis in facilitating the diagnosis in either case 2 or case 3? Genetic linkage studies could be attempted to clarify the status in case 2, in which there was an affected mother, two other affected siblings, and the one unaffected sibling in the mother's generation. The family of case 3 does not fulfill standard criteria for genetic linkage analysis, since there are no family members who definitely have ADPKD (see chapter 11). Thus, in some children (as in case 3), a definite diagnosis of ARPKD or ADPKD cannot be established, careful follow-up will provide the most useful information regarding prognosis.

It is reasonable to ask if there are any epidemiological factors that predispose an at-risk child to the diagnosis of ADPKD in childhood. The age of children with ADPKD ranges from fetal life to 18 years of age [25, 27–29]. The pedigree displayed in figure 12-1 raises another aspect of diagnosis in childhood. All the subjects in the third generation were examined because of family history of ADPKD not because of clinical complaints.

The sex of the affected parent may have an influence on the age when clinical manifestations appear. A precedence for this exists in another autosomal dominant disorder, Huntington's disease, in which off-spring of affected fathers have earlier onset of disease than offspring of affected mothers [33]. Likewise, data from the study of Sedman et al. suggested that children of affected fathers had an earlier onset of ADPKD [29]. However, other studies have not demonstrated a difference in age at onset related to the sex of the affected parent [30]. Large prospective screening studies will be required to answer this question, It is interesting to speculate that families such as those shown in figure 12-1 represent circumstances in which both parents have

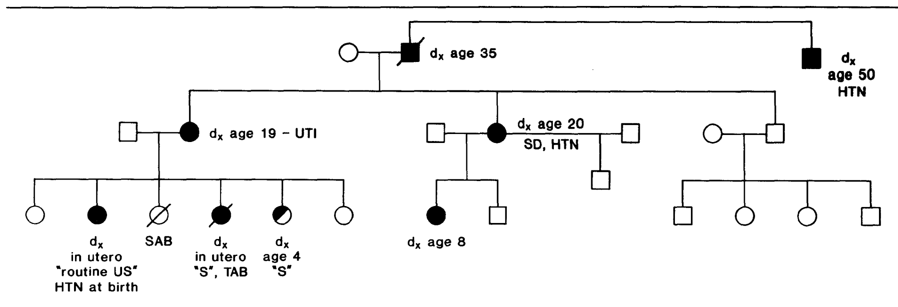

Figure 12-1. The pedigree of an ADPKD family with variable age. d_x = age of diagnosis; SD = stone disease; UTI = urinary tract infection; HTN = hypertension; SAB = spontaneous abortion; TAB = therapeutic abortion; "S" = suspicious for ADPKD unilateral cysts; US = ultrasound.

Table 12-4. Signs and symptoms of ADPKD in children

Abdominal mass
Abdominal or flank pain
Headaches
Urinary tract infection
Hematuria
Hypertension

ADPKD, and hence two copies of the allele produce early-onset disease. However, in this family and in a few others studied, this was not the case [34]. It is also possible that there are modifying alleles at the ADPKD gene locus that influence clinical presentation [35]. Although it appears that the ARPKD locus is not at or near the ADPKD-1 site [36], it has been suggested, but not proven, that those individuals with early onset have both the ADPKD gene and ARPKD gene [34].

What are the clinical manifestations of ADPKD in children?

Neonates and infants often present with abdominal masses [27–30]. However, only about 30% of children with ADPKD appear to come to the attention of the physician because of clinical complaints or abnormalities [29]. The presenting signs and symptoms are hypertension, proteinuria, hematuria, or palpable abdominal mass [27–30]. A majority of children are identified in family screenings. However, when carefully questioned, 74% of the children appear to have signs compatible with ADPKD (table 12-4) [29]. Moreover, on examination approximately 20% of ADPKD children have a blood pressure that exceeds the 95% confidence limits for blood pressure in their age group [27, 29]. A majority of ADPKD children have an abnormal urinalysis

manifested by hematuria, pyuria, or proteinuria [27–29]. However, the abnormalities on the urinalysis may be minimal with only 1–5 WBC/hpf or 1–5 RBC/hpf [29]. Pyuria or hematuria of more marked degree may only occur in 5% to 10% of children on any isolated urinalysis [29].

The frequency of extrarenal manifestation of ADPKD has not been systematically evaluated in children. However, as stated above, hepatic cysts appear to be very uncommon. Although there are no systematic data regarding berry aneurysms in the young, one of 14 patients with presumed ARPKD developed signs of a berry aneurysm with subsequent diagnosis and surgical repair at 15 years of age [26]. It is interesting to speculate that this child may, in fact, have ADPKD. In addition, a girl from an ADPKD family who had presented at age two with an abdominal mass died of a ruptured berry aneurysm at age 12 [13].

What is the natural history of ADPKD in children?

Obviously, the natural history of ADPKD in children is of critical interest to anyone counseling these children and their families. In many of the early reports, children with ADPKD died of renal failure at an early age [10, 18, 19, 24]. However, generalizations from isolated reports of a common disorder are fraught with the danger that only the worst examples of the disorder may be published. This appears to be the case in this instance. Eighteen children with ADPKD were followed by Sedman et al. for a mean of 7.6 years. Only 17% progressed to end-stage renal disease during follow-up at ages 3, 15, and 39 years [29]. An additional 27% had developed hypertension and/or worsening renal function [29]. Although there is a suggestion that children who present in the first year or life with a clinical abnormality may have a worse prognosis [29], Cole has found that during a mean follow-up 31.9 ± 15 months such children did well [27].

What is appropriate management of ADPKD in children?

Children with ADPKD should be in the care of a physician who is familiar with the manifestations and natural history of the disorder and is cognizant of the treatment of these manifestations. This is critical in order to convey an appropriately optimistic outlook to the patient and family and to provide optimal care. Children, particularly those diagnosed in a screening setting, should not have limitations placed on any aspect of daily living. There may be some concerns about children with large cysts or large kidneys developing ruptured cysts or hematuria by engaging in contact sports. However, there are no specific data to demonstrate this complication. Certainly, the concern does not seem warranted in children with normal-size kidneys and small cysts. Given that pyuria and hematuria occur in ADPKD, these abnormalities should not be equated with infection in the absence of documentation by urine culture. Blood pressure should be carefully followed. Although there are no formal data on the effect of normalization of blood pressure from an early age,

it appears reasonable to attempt to achieve this. It would appear appropriate to lower the blood pressure level to the 75th percentile or below for age-matched subjects [37]. Such an aggressive therapeutic approach is based on the apparent relationship between hypertension and disease severity in adult ADPKD patients [44]. The physician should consider having the parent learn to take the blood pressure and maintain a log. If a child develops azotemia, he or she should be treated as are other children with renal insufficiency.

What children in ADPKD families should be evaluated for ADPKD?

The need to evaluate a child who comes to the attention of a physician because of a complaint or finding of abdominal mass, hypertension, or proteinuria is apparent. The guidelines for evaluating asymptomatic children at 50% risk for ADPKD are less clear. Random screening does not appear warranted in the absence of specific disease prevention interventions. However, all children who are at 50% risk should be seen by their physician yearly for blood pressure determination. Children in unusual clinical circumstances, such as those who are members of a family with a history of early rupture of berry aneurysm or those in whom therapy would be instituted if ADPKD were present, should be evaluated for the disorder. An example of the latter circumstance would be treatment of borderline hypertension in a child with ADPKD. Abdominal ultrasonography is the diagnostic tool of choice at the present time. It can be performed with relative ease in even young children; it requires no contrast or radiation exposure; it can detect cysts in children, and it requires no assessment of the entire family. If renal cysts are present, the diagnosis is confirmed. If renal cysts are not present and a diagnosis is needed, genetic linkage can be performed if the family pedigree is adequate for this methodology.

AUTOSOMAL POLYCYSTIC KIDNEY DISEASE IN ADULTS

Although the answers to the previously noted five questions are somewhat different in the adult population, the same issues deserve attention.

What clinical information is necessary to establish the diagnosis?

The question of ADPKD in an adult arises either in screening a member of a ADPKD family who wishes to know if he or she is affected or in evaluating a patient with signs or symptoms compatible with ADPKD. The data that can be utilized in establishing the diagnosis include family history, personal history, physical examination, routine laboratory data, imaging studies, and gene linkage studies. Family history is positive in only about 50% of subjects [3]. This relatively low percentage of family history does not appear to reflect a high spontaneous mutation rate, but rather inadequate information. Greater public education about the disease and earlier diagnosis as a result of readily

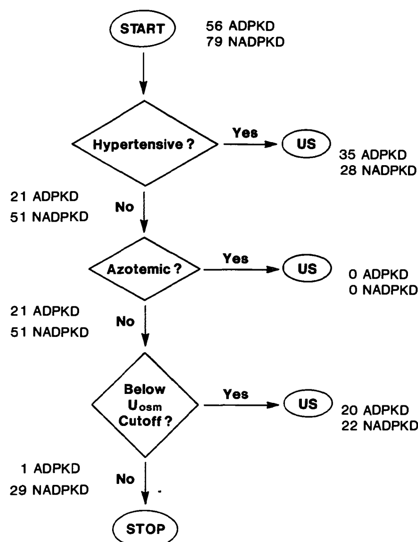

Figure 12-2. An algorithm developed from a population of 56 ADPKD and 79 NADPKD subjects. The decision points are diamonds. US represents ultrasound of the kidneys. The number of subjects identified at each decision point is listed. The Uosm cutoff value is derived from normal, nonazotemic, normotensive family members [39]. Source: Gabow PA, Kaehny WD, Johnson AM, Duley IT, Manco-Johnson M, Lezotte DC, Schrier RW: The clinical utility of renal concentrating capacity in polycystic kidney disease. Kidney Int 35:678, 1989.

available sensitive imaging techniques will undoubtedly increase the frequency of positive family histories. In fact, in 90% of cases other family members with ADPKD are identified when sensitive imaging techniques are employed. Neither history, physical examination, nor routine laboratory data are sufficiently sensitive or specific to definitely establish or exclude the diagnosis [38]. In the setting of screening at-risk family members, a clinician can utilize a sequential algorithm that will identify approximately 97% of affected family members and correctly exclude 20% to 30% of unaffected family members, making further evaluation unnecessary in the latter group (figure 12-2) [39]. The algorithm utilizes a logical sequence of patient evaluation, with high-risk subjects proceeding to imaging studies. All at-risk individuals with hypertension require further evaluation, since 60% of individuals with ADPKD are hypertensive [38]. Normotensive individuals proceed to the next branch point, namely determination of serum creatinine concentration. Any member of an ADPKD family with abnormal renal function requires further definition of the cause of the dysfunction, with the most appropriate first diagnostic study being renal imaging. Those normotensive individuals with normal renal function then undergo testing of renal concentrating ability. This is accomplished with 12 hours of fluid and food deprivation followed by voiding and the subcutaneous administration of five units of aqueous vasopressin. Urinary

osmolality is then measured on the next spontaneously voided urine. If the value is equal to or above the value considered normal for age-matched normotensive subjects, the subject is considered to have normal concentrating ability and requires no further evaluation [39]. However, the algorithm yields a false negative rate of 3% [39]. Thus, at-risk individuals desiring a more definitive assessment should undergo imaging studies or gene linkage analysis.

Patients who are being evaluated for ADPKD in response to a clinical complaint or abnormality should undergo imaging studies since these may elucidate the abnormality responsible for the complaint. The imaging modality of choice for screening is ultrasonography, since it is sensitive and does not expose the individual to radiation or contrast media. There appears to be no advantage in using either computed tomography with contrast or magnetic resonance imaging studies for routine screening. However, there may be a role for computed tomography in assessing certain presumed complications of ADPKD, such as cyst hemorrhage or stone [40].

Should gene linkage analysis be employed rather than abdominal ultrasonography for screening an adult individual who is at 50% risk? Gene linkage studies require other family members to be involved, which is not demanded by ultrasonography (see chapter 11). Linkage analysis provides data on a statistical likelihood of the gene carrier state, whereas a positive ultrasonographic study provides certainty of gene status. In addition, a positive ultrasonogram provides data on the extent of structural alterations present in the kidney as well as in the liver if complete abdominal ultrasonography is performed. When the gene itself is isolated, testing will then provide exact confirmation rather than probability information, and testing of other family members will not be necessary to establish the diagnosis in a given individual. However, even then only imaging techniques will provide information on the presence or severity of structural alterations and the presence of renal complications of ADPKD. The gene linkage is most valuable in an individual whose ultrasonography is nondiagnostic or normal and who wishes further clarification of his or her gene status, or in such an individual whose management would be altered by the clinician's knowledge of the subject's status. An example of the former circumstance would be an individual whose family planning would be modified by knowledge of gene status. An example of the latter circumstance would be the case in which the clinician would treat borderline hypertension in an affected subject but would not do so in an unaffected family member. Fortunately, in a majority of adults over 30 years of age the diagnosis is easily established by the presence of large kidneys, with multiple renal cysts distributed throughout the renal parenchyma. If the renal cysts are visualized by ultrasonography, computed axial tomography with intravenous contrast, or magnetic resonance imaging, it is likely that ADPKD is the diagnosis. Differentiation of ADPKD from multiple simple cysts (table 12-5) [41] and other renal cystic disorders such as tuberous sclerosis [42] is generally not difficult in this age group with current imaging techniques. Since

Table 12-5. Characteristics of renal cystic disorders (adapted from ref. 41).

Feature	Simple cysts	ADPKD
Inheritance pattern	None	Autosomal dominant
Prevalence	Common, increasing with age	1/400 to 1/1000
Age of onset	Adult	Usually adults
Presenting symptoms	Incidental finding: hematuria	Pain, hematuria, infection, family screening
Hematuria	Occurs	Common
Recurrent infections	Rare	Common
Renal calculi	None	Common
Hypertension	Rare	Common
Method of diagnosis	Ultrasound	Ultrasound
Renal size	Normal	Normal to very large

tuberous sclerosis can have renal cysts as the sole manifestation, this possibility should be consider and the presence of adenoma sebaceum, retinal lesions, ash leaf macules, port wine hemangiomas, or subungual fibromas should be sought during routine examination. Similarly, the presence of the extrarenal manifestations of ADPKD should be sought on examination, since their presence always strengthens the diagnosis.

What are the clinical manifestations of ADPKD?

As many as 32% of the young population of ADPKD patients may be asymptomatic. The asymptomatic individuals are younger than those individuals with symptoms (27.3 ± 2.1 versus 38.1 ± 1.4 years) [38]. The complaints are diverse and could result in symptomatic individuals presenting initially to a variety of medical specialists. However, the most common complaints of flank, abdominal and back pain, and hematuria appear to relate to the kidneys. The exact mechanisms producing these manifestations have not been elucidated, but some patients appear to experience dramatic relief of pain with surgical decompression of the renal cysts [43]. Hypertension is very common in ADPKD, occurring in approximately 60% of individuals prior to the onset of azotemia [38]. The relationship of the severity of the renal cyst development and the occurrence of hypertension suggest that this symptom is also a consequence of the renal lesion [44]. Surprisingly, headaches are another very common clinical problem, relating more to the presence of ADPKD than to the often present hypertension [38].

The systemic nature of the disorder is reflected in the other clinical manifestations. Hepatic cysts occur in 38% to 63% of ADPKD patients [45–47]. Those individuals with hepatic cysts are older than those without hepatic cysts (42.3 ± 2.0 versus 32.5 ± 1.4 years) [38, 47]. In addition, subjects with renal insufficiency appear to have hepatic cysts more commonly than ADPKD subjects with normal renal function [47]. Moreover, prior to age 50, women have a higher prevalence of hepatic cysts than men [48]. Although the liver

is a frequent site of structural disruption in ADPKD, this often impressive structural abnormality is rarely associated with portal hypertension or clinically apparent alteration in hepatic function [45]. Available information suggests an association between ADPKD and diverticulosis and/or diverticulitis [49, 50]. Further studies will be required to define this possible association.

Individuals with ADPKD often complain of palpitations, have systolic murmurs and clicks, and manifest a 26% prevalence of mitral valve prolapse [51]. The most devastating nonrenal manifestation of ADPKD is rupture of a berry aneurysm. Although the association of this catastrophic complication with ADPKD hass been noted by a number of investigators, the frequency of berry aneurysm in ADPKD has varied from 10% to 40% [52–55]. Preliminary data suggest that berry aneurysms may cluster within certain ADPKD families [56].

What is the natural history of ADPKD in the adult?

Discussions regarding the natural history of ADPKD are in essence discussions of the natural history of the renal disease, since there is little to no information regarding the natural history of extrarenal manifestations of the disorder. Less than 10% of the nephrons appear to be directly involved with cystic changes [57]. Thus, it is surprising that end-stage renal disease should ever occur. Nonetheless, as many as 50% of ADPKD subjects progress to renal failure [4]. The mechanisms that may participate in this progression include cystic compression with progressive fibrosis of noncystic parenchyma, hyperfiltration by unaffected nephrons, and hypertension-induced renal damage. There is a paucity of data regarding these hypothesized mechanisms. Electron microscopy of a kidney of a person with early ADPKD did show compression of adjacent nephrons [57]; mathematical calculations dependent upon the growth of spheres are compatible with the hypothesized effect of compression of adjacent nephrons [58]. There is little information pertaining to hyperfiltration in ADPKD [58a]. There are no longitudinal prospective studies evaluating the effect of treating hypertension in ADPKD, but there are data that indicate that normotensive subjects experience better patient and renal survival than do hypertensive ADPKD subjects [59]. Circumstantial data also implicate hypertension as an important factor in the progression of renal disease. In Dalgaard's classic study performed prior to the availability of effective antihypertensive therapy, end-stage renal disease occurred in a majority of individuals at an average of 50 years of age [4]. A more recent study conducted during the time period of anti-hypertensive therapy revealed that 52% of subjects at 70 years of age were alive without renal replacement therapy [60]. In addition, Igelais et al. demonstrated better 10-year renal (92% versus 48%) and patient (80% versus 40%) survival in ADPKD subjects diagnosed after 1956 than in those diagnosed prior to 1956 [59]. Moreover, patients diagnosed before age 35 appear to have a better renal prognosis than those diagnosed after age 35 [59]. It has been suggested that these improved survival statistics may reflect the

result of early treatment of hypertension. The role of hypertension in both the progression of the renal disease and the rupture of berry aneurysms requires further study. There is great variability in the length of time to end-stage renal disease among individuals with ADPKD, with one child requiring renal transplantation at age 3 and some adults maintaining normal renal function until past age 70. The speculation has been raised that there are different rates of progression of renal disease in different families [4, 61]. If such differences were clearly demonstrated, factors such as hypertension and dietary habits (particularly protein intake), as well as genetic factors, would need to be delineated. Not only must more effort be expended in understanding the natural history of the renal aspects of ADPKD, but also efforts must be made to gain some insight into the natural history of the hepatic abnormalities, the berry aneurysms, and the cardiac valvular abnormalities. Although hepatic insufficiency or portal hypertension is rare in ADPKD, 3% of ADPKD subjects may have serious hepatic cyst infections, in some instances resulting in death [45]. Some individuals with ADPKD do require mitral valve replacement [62]. However, the frequency and risk factors for this complication remain to be defined.

What is the appropriate management for ADPKD is adults?

The first aspect of treatment of any slowly progressive, late-onset hereditary disorder is education of the patient and the at-risk family members about the inheritance, clinical manifestations and natural history of the disorder. Affected individuals should be aware that this is not only a renal disorder but also a systemic disorder. Even in the absence of a prospective study, normalization of blood pressure should be a therapeutic goal. Symptoms of urinary tract infection should be pursued with urine cultures, and if infection is present prompt therapy should be instituted.

The patient with presumed pyleonephritis who does not promptly respond to therapy usually appropriate for pyleonephritis should be considered to have renal cyst infection and should be treated with an antibiotic that exhibits cyst penetration. Lipid solubility is apparently an important characteristic in the penetration of cyst epithelium [63]. Thus, the antibiotics of choice for presumed cyst infection include trimethoprim-sulfamethoxazole [64], chloramphenicol [65] and ciprofloxacin [66]. Patients should be educated regarding the warning signs of ruptured berry aneurysms. A patient with a strong family history of ruptured berry aneurysm or a patient whose occupation or avocation would put himself or others at grave risk in event of rupture (e.g., airplane pilot) should be considered for evaluation of cerebral circulation. This may be accomplished by high-resolution computed tomography [67] or by angiography. An affected individual with a regurgition murmur should be considered for cardiac echocardiography to determine if mitral valve prolapse is present. In such a circumstance, antibiotic prophylaxis for endocarditis should be considered [68]. This diagnosis also needs to be entertained in

affected individuals with atypical chest pain, supraventricular arrhythmias, or central nervous system thromboembolic events [69].

Pain is a common problem in ADPKD [38]. Often patients require large amounts of analgesics to control this troublesome symptom. The effect of such analgesic use on renal function has not been assessed. Recently, Bennett et al. have demonstrated that some patients responded to surgical de-roofing of cysts with dramatic reduction of pain [43]. This intervention deserves consideration in patients with disabling pain.

Which family members should be evaluated for ADPKD?

Random, nonclinically directed screening of at-risk subjects does not appear warranted at the present time [70]. Any screening that is performed should be part of a complete evaluation and appropriate counseling [70]. Individuals who are at risk for ADPKD and present to a physician with signs or symptoms related to ADPKD should be evaluated in order to establish the diagnosis and define the reason for the clinical problem. Asymptomatic adult subjects at 50% risk should be considered for screening evaluation if they wish to know their genetic and clinical status. This screening can be performed as outlined above. Asymptomatic individuals who are in families with a high frequency of berry aneurysms should be encouraged to have their status defined, since if they are positive it appears appropriate to screen them for aneurysms. If they are not positive, such screening would not be indicated [71]. Affected individuals who are planning a pregnancy, are pregnant, or whose spouse is pregnant should be informed that technology exists for determining the gene status of the fetus in some families [72]. They should also be informed that establishing high likelihood of the ADPKD gene in the fetus does not provide a prognosis for the natural history of the disorder. Moreover, it must be clear that ADPKD is not a disorder that fulfills proposed criteria for third-trimester abortion [73].

Thus, ADPKD is a common genetic disease, presenting in both adults and children. Knowledge about the methods of diagnosis, clinical features, and course of this disorder provides direction for the clinician who is caring for and counseling adults and children with ADPKD and their families.

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13. AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE

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The age distribution of cystic kidneys has two peaks, one in infancy and the other in adulthood [1]. Patients who present in infancy with bilateral cystic kidneys can be subdivided further into a group with heterogenous conditions (e.g., Meckel syndrome), in which the renal abnormality can best be described as cystic renal dysplasia, and a second group with large, spongy, polycystic kidneys without any evidence of dysplasia. The occurrence of polycystic kidneys in more than one newborn in a sibship was noted by Potter [2], and an autosomal recessive mode of inheritance was suggested by Lundin and Olow [3] and Heggo and Natvig [4]. Blyth and Ockenden [4] proposed that there were four discrete groups of patients under the rubric of *polycystic kidneys and liver*. However, reports that have documented, during the newborn period, polycystic kidneys in one sib and congenital hepatic fibrosis (CHF) and renal tubular ectasia in another, cast doubt on this formulation [6]. A careful analysis of reported cases and a detailed reevaluation of the Great Ormond Street experience [7], including many of the patients originally reported by Blyth and Ockenden [4], have led to the conclusion that the various phenotypic expressions of autosomal recessive polycystic kidney disease (ARPKD) and congenital hepatic fibrosis are the result of an abnormality of a single allelic change, rather than the result of four or five different mutations [5].

INFANTILE POLYCYSTIC KIDNEY DISEASE VERSUS POLYCYSTIC KIDNEY DISEASE IN INFANCY [8]

Conditions with similar clinical features to those of ARPKD that may present in infancy are listed in table 13-1. In the *lingua franca* of medicine, autosomal

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Table 13-1. Infantile polycyctic kidney disease versus polycystic kidney disease in infancy [8]: Differential diagnosis of autosomal recessive polycystic kidney disease

1. Autosomal recessive polycystic kidney disease
2. Autosomal dominant polycystic kidney disease
3. Polycystic kidneys associated with tuberous sclerosis
4. Cystic dysplasia in Meckel syndrome
5. Jeune's asphyxiating thoracic dystrophy
6. Ivemark syndrome
7. Familial hypoplastic glomerulocystic kidney
8. Congential hypernephronic nephromegaly with tubular dysgenesis

recessive polycystic kidney disease is more commonly, and less accurately, known as infantile polycystic kidney disease. Similarly, autosomal dominant polycystic kidney disease (ADPKD) is usually referred to as adult polycystic kidney disease. These are clearly misnomers, because the so-called "adult" form can be detected in utero [9] and can present in infants [10–13] and children [14], and the so-called "infantile" form can present in adolescents and occasionally even in adults [15].

Although ARPKD and ADPKD are two completely different conditions with renal cysts in common, it is surprising how difficult it can be to differentiate one from the other in some individuals [16]. There are no DNA probes for the precise diagnosis of ARPKD. Before the advent of DNA probes for the accurate diagnosis of ADPKD [17–19], it was difficult to make a precise diagnosis in some cases in the absence of an informative pedigree, despite the use of clinical, radiologic, ultrasound, and histologic criteria. A definitive diagnosis could be made only after examination of the entire kidney by gross and microscopic techniques. Hepatic involvement with portal fibrosis and proliferation of bile ductules has been considered to be one of the most important ways of differentiating between the recessive and dominant types of polycystic kidneys [16]. It has generally been assumed that the presence of congenital hepatic fibrosis in patients with ARPKD, and its absence in ADPKD, serves as an important differentiating feature between these two conditions, especially when the pedigree is noninformative. ARPKD is almost always associated with hepatic fibrosis [20], whereas CHF was not considered to be a feature of ADPKD [21]. Cysts are frequently noted in the livers of adult patients with ADPKD [22] and are usually associated with Von Meyenburg plexuses within or close to portal tracts, but do not communicate with bile ducts [23]. Occasionally, the cysts may cause portal hypertension, and this may be complicated by upper gastrointestinal hemorrhage from bleeding esophageal varices [24]. Unfortunately, however, fibrosis of the portal tracts (biliary fibroadenomatosis) has been described in patients wth unequivocal features of ADPKD in adults [23] and children [24, 25].

Infants with tuberous sclerosis may present with seizures and enlarged kidneys and similar appearances to those opf ADPKD on radiologic and ultra-

sound examinations [26–28]. This condition can be differentiated from ARPKD if one or more of the other features of tuberous sclerosis are present: ash leaf depigmented spots confirmed by examination with ultraviolet light, evidence of tubers in the brain by CT scan examination, leiomyomas in the heart or kidney, or a similarly affected parent. The inheritance is autosomal dominant.

Differentiation of ARPKD from Meckel syndrome is not difficult if there is full expression of the latter [29]. The presence of postaxial polydactyly, microphthalmia, and an encephalocele in an infant with enlarged, cystic kidneys and hepatic fibrosis confirms the diagnosis of Meckel syndrome. Partial expression of these features can occur, even within families. In Meckel syndrome, renal cortical spherical cysts increase in size toward the medulla and are separated from one another by interstitial stroma. This appearance is different from that seen in ARPKD, in which the dilated tubules are arranged in an orderly fashion at right angles to the capsule of the kidney [30]. The inheritance of Meckel syndrome is autosomal recessive. Prenatal diagnosis is possible.

Jeune syndrome [31] is characterized by the occurrence in infancy of narrow thorax with short ribs, as well as respiratory failure, short stubby hands, fingers with cone-shaped epiphyses, and trident-shaped iliac bones. Survivors develop postnatal short-limbed dwarfism with metaphyseal dysplasia [32]. Renal involvement in infancy consists of cortical cysts or cystic dysplasia. The renal lesion found in older children is indistinguishable from that seen in juvenile nephronophthisis [32]. Patients with Jeune syndrome also have features of congenital hepatic fibrosis with a spectrum of severity [32]. Similarities between ARPKD and Jeune syndrome include the occurrence of extreme degrees of pulmonary hypoplasia and rib abnormalities (in some neonates with ARPKD), cystic kidneys, and hepatic fibrosis. However, these conditions can be differentiated from each other by the occurrence of the other bone abnormalities in Jeune syndrome. This condition is inherited as an autosomal recessive trait.

There are very few reports of patients with Ivemark syndrome of renal–hepatic–pancreatic dysplasia [33, 34]. Patients with renal–hepatic–pancreatic dysplasia do not appear to form a homogenesis entity [34]. These infants have severe respiratory distress at birth and are found at postmortem examination to have cystic kidneys, hepatic fibrosis, and pancreatic fibrosis. The appearance of the cystic changes in the kidneys differs from that seen in ARPKD in that there is cystic dysplasia, with abnormal duct differentiation, deficient nephron differentiation, and glomerular cysts [34]. The inheritance seems to be autosomoal recessive.

Familial hypoplastic glomerulocystic kidney [35] may be difficult to differentiate by ultrasonographic studies from ARPKD [36]. This condition is inherited as an autosomal dominant trait and is characterized by onset of chronic renal failure in infancy, the demonstration of cystic kidneys by renal ultrasound examination, and histologic evidence of glomerular cysts and

small, primitive glomeruli. Small, smooth kidneys with simple collecting systems are demonstrated by intravenous urography. The liver is normal [37]. Renal function remains normal [38] or stable [35, 39] over a period of many years.

Some infants with ARPKD are stillborn with nonfunctioning lungs, and others may die soon after birth with large nonfunctioning kidneys; their condition thereby resembles the entity of congenital hypernephronic nephromegaly with tubular dysgenesis [40–42]. There are features of the oligohydramnios sequence with Potter facies and hypoplastic lungs. The kidneys are enlarged and do not function. There is an increased number of glomeruli, tubules are immature and lack proximal convolutions, and there is interstitial fibrosis [41]. The inheritance seems to be autosomal recessive.

CLINICAL FEATURES OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (ARPKD); ARPD; INFANTILE POLYCYSTIC KIDNEY DISEASE

There are two main clinicopathologic phenotypes of ARPKD. [20]. Each is related mainly to the age of presentation of the patient. Renal disease predominates over liver involvement in patients who present at birth or in the neonatal period. These patients correspond with the *perinatal, neonatal,* and *infantile* forms of Blyth and Ockenden [5]. Patients who present in childhood or adolescence tend to do so with predominantly hepatic findings and less obvious kidney manifestations. These correspond to the juvenile group of Blyth and Ockenden [5]. Because of these two different phenotypic manifestations, it is useful to discuss them separately under the headings of *autosomal recessive polycystic kidney disease with congenital hepatic fibrosis* and *congenital hepatic fibrosis and renal tubular ectasia.* This may be an artificial delineation that does not imply that there are two discrete forms of the condition, but it acknowledges our understanding that there are main phenotypes.

Autosomal recessive kidney disease with congenital hepatic fibrosis

Severely affected neonates who have the oligohydramnios sequence (Potter facies, limb abnormalities, and severe pulmonary hypoplasia) die at or soon after birth. However, death does not occur in all infants who present at birth with oligohydramnios, hypoplastic lungs, and large kidneys. Respiratory distress is usually the main problem in patients who present at or close to birth. Prior to the use of artificial respiratory support, many died in the newborn period. The condition often is first suspected because of an abdominal or flank mass [16].

Anuria or oliguria are usually noted in infants whose pulmonary function is extremely compromised. With improvement of the respiratory complications, however, urine output tends to increase and azotemia tends to improve. Patients who survive the neonatal period usually have decreased glomerular filtration rates [44] but manage to maintain sufficient function into adolescence. The ability to concentrate the urine is impaired, and there is a

tendency to a mild metabolic acidosis and reduced net acid excretion [44]. The younger the age at presentation, the more abnormal are these tests of renal function [44]. Hypertension can be a major problem [7, 16, 43] that initially may be associated with hyponatremia [7]. Peripheral renin activity and serum aldosterone levels are almost always within the normal range. Although the hypertension may respond to treatment with an angiotensin-converting enzyme inhibitor, hyperreninemia does not seem to be the cause of the elevated blood pressure levels [7]. A more likely explanation is that these patients have been volume-expanded following administration of intravenous fluids, and are unable to excrete the fluid optimally because of a decrease in glomerular filtration rate and an inability to dilute the urine maximally [7]. Support of this hypothesis stems indirectly from the observations that urine sodium concentrations are low, that urine osmolality is relatively high despite low serum osmolality, and that furosemide administration helps to normalize the serum sodium concentration and the blood pressure [7].

Congenital hepatic fibrosis and renal tubular ectasia

Patients who present in childhood or adolescence tend to do so with the complications of hepatic fibrosis and have firm livers and splenomegaly. Hematemesis from bleeding esophageal varices caused by portal hypertension is a frequent and distressing feature. Alvarez et al. [45] studied 27 children with congenital hepatic fibrosis, most of whom had abnormal kidneys with renal tubular ectasia or polycystic disease. Esophageal varices were seen by endoscopy in 21 of 27 patients, and 11 of these had hematemesis. None had abnormal liver function tests. Cholangitis occurred in some cases [45]. Many patients have growth retardation. Anemia, leukopenia, and thrombocytopenia are usually found and are the consequences of hypersplenism.

RADIOLOGIC FEATURES OF ARPKD

In the affected neonate the kidneys are relatively larger than in older children. Cysts are rarely seen on excretory urograms. The degree of function noted by excretory urography is commensurate with the severity of renal impairment. Typically, the nephrogram has a mottled or streaky appearance from pooling of contrast medium in dilated cortical and medullary cysts [46]. Linear opacifications may also be seen as a result of retention of contrast medium in dilated medullary collecting ducts [46]. An incorrect diagnosis of ARPKD may be made in some infants on the basis of bilaterally enlarged kidneys, prolonged nephrogram, and alternating radiodense and radioluscent streaks radiating from medulla to cortex [47]. The cause of this transient nephromegaly in not known.

Some patients diagnosed as having congenital hepatic fibrosis and the radiologic features of medullary sponge kidney [48, 49] actually have renal tubular ectasia and CHF [50] and therefore have the same genetic defect as those with ARPKD. The radiologic features of medullary ductal ectasia are similar to

those seen in patients with medullary sponge kidneys, clinically different condition that does not seem to inherited.

RENAL ULTRASOUND FEATURES OF ARPKD

The sonographic appearances of the kidneys in patients with ARPKD depend to a large extent on the age of the patient. Garel [51] has defined three patterns:

Pattern I: Massive kidney enlargement
Increased echogenicity of the entire parenchyma
Loss of corticomedullary differentiation
Loss of central echo complex
Small macrocysts, below 2 cm in diameter

Pattern II: Massive kidney enlargement
Increased echogenicity, mainly in the medulla
Macrocysts remain below 2 cm

Pattern III: Moderate kidney enlargement
Increased echogenicity confined to medulla
No macrocysts seen

Pattern I is seen in neonates and infants, whereas patterns II and III tend to be observed in older children [51]. Renal ultrasonography cannot distinguish with certainty between ARPKD and ADPKD [13, 51, 52]. Ultrasound has been used to diagnose ARPKD in utero. In a prospective study, 10 of 19 at risk had ARPKD [52]. There were no false-positive diagnoses, but the diagnosis was missed in one mildly affected fetus. Each affected fetus had hyperechogenic kidneys. Oligohydramnios, an absent bladder, and enlarged kidneys were often noted [52]. Most affected cases were not apparent before 24 weeks.

PATHOLOGIC FEATURES OF ARPKD [53)

The kidneys are enlarged, spongy in appearance, and in contrast to ADPKD, remain reinform. The predominant changes are those of dilated collecting ducts that are neatly arranged perpendicular to the surface of the kidney. There are no dysplastic elements. The dilated tubules have been proven to be collecting ducts by microdissection studies [4, 54] and by binding with peanut-derived lectin [55].

The liver is always involved in ARPKD. There is, to our knowledge, no documented case of ARPKD in which the liver has been entirely normal. That is, there are no meticulous studies in which a patient with polycystic kidneys, whose sib(s) had ARPKD with hepatic fibrosis, had a liver that was histologically normal. One cited exception [53] deals in a superficial and perfunctory way with the subject of liver involvement; a reevaluation of the histologic specimens of these patients in the light of current knolwedge of liver involvement in ARPKD would be of great interest [56]. In affected patients, with portal areas are enlarged by an increased number of dilated

Table 13-2. Congenital hepatic fibrosis (CHF): Associations [21,71,72]

CHF and polycystic kidneys
Autosomal recessive polycystic kidneys (or, renal tubular ectasia)
Autosomal dominant polycystic kidneys [23,24,25]
CHF and hereditary tubulo-interstitial nephritis
Juvenile nephronophthisis [32,72,73]
Juvenile nephronophthisis with tapetoretinal degeneration [74]
Biedle–Bardet syndrome [75]
CHF and cystic kidneys, colobomata, encephalopathy [76]
Asphyxiating thoracic chondrodystrophy [31,32]
CHF and hereditary renal dysplasias
Meckel syndrome [29]
Chondrodysplasia syndromes [21,71]
Renal–hepatic–pancreatic cystic dysplasia [33,34]
Zellweger syndrome [77]

bile ductules, which are surrounded by fibrous tissue. The dilated ductules rarely become truly cystic. The liver cells are normal. There may be evidence of cholangitis in some, especially older, patients. The pathology of the liver in infants with ARPKD may appear sufficiently different from that of adolescents with CHF and renal tubular aectasia to imply, as some have, that these are two different conditions [57]. It would seem, however, that these are age-related differences or variations in phenotype, rather than expressions of two different genetic disorders.

Many, but not all, patients with CHF have evidence of abnormal kidneys, and the types of renal involvement encompass a large number of developmental disorders of the kidneys, most of which are inherited [table 13-2).

PROGNOSIS OF ARPKD

There are a few studies on the long-term prognosis of ARPKD. Lieberman et a1. [20] were the first to note that not all patients with ARPKD died at birth. This was in contrast to the outcomes reported by Blyth and Ockenden [5], who noted that 15 of 16 neonates with ARPKD died before six weeks of age. More encouraging results were reported by Thomson and Isdale [58]: 10 of 18 of their patients who presented before a week of age died, whereas 11 out of 12 who presented after one week lived. Kaplan et al. studied 55 patients with ARPKD who had been seen at Great Ormond Street Hospital, London, from 1950 to 1985 [7]. Included among these were many of the patients of Blyth and Ockenden [5]. Fourty-eight of these patients could be traced, 24 were known to have died, and 31 were examined or contacted between 1980 and 1985. Twelve of 24 patients who presented in the neonatal period survived beyond two years of life. The probability of survival was assessed by actuarial analysis. If survivial at 12 months was taken as 100%, then the estimated probability of survival beyond 15 years was 78%.

GENETICS OF ARPKD

The inheritance of ARPKD is by a classical Mendelian autosomal recessive mode: the parents are unaffected, and the recurrence risk is 25% in each pregnancy. In many patients the diagnosis is relatively straightforward, especially if a sib has been shown to have the condition, or if the patient has presented in the newborn period with respiratory distress and very large kidneys, and has typical ultrasonographic, renal histologic, and hepatic features of ARPKD. However, there may be variable expression within a sibship.

Earlier reports stressed that the apparent intrafamilial concordance in the age of onset of illness of sibs [5, 59] was indicative of genetic heterogeneity of ARPKD: the concordance suggested that there were several causes, with four or five mutant genes being responsible for closely related, but phenotypically different entities [60]. Families in which children of different ages were affected were referred to as intermediate forms and were considered to be compound heterozygotes resulting from two abnormal or mutant genes [60]. These studies were based on carefully constructed pedigrees, and patients were included only if there was a histologic diagnosis [5]. The assignment of patients with *polycystic kidneys and liver* in childhood to one of four groups (perinatal, neonatal, infantile, and juvenile) was based on the age of presentation and depended in some cases on prior awareness of the condition in a previously affected sibling. These four groups were thought of as entirly separate entities. The argument for accepting a classification derived from the age of presentation of patients in a family was supported by the fact that the clinical and histologic phenotypes at each extreme (perinatal versus juvenile) were entirely different. Young patients had serve renal involvement and subclinical changes in the liver; on the other hand, the older the patient was at presentation, the more severe were the manifestations of liver disease, and the less important was the kidney disease.

These views are still accepted widely [60–62] because the phenotypic expression in so many kindreds has been so consistently similar. A dramatic example is that of the family reported by Lathrop [63]. Four of seven children had *cystic disease of the liver and kidney*. One of these four was assymptomatic, but had radiologic evidence of renal tubular ectasia; the others had portal hypertension that manifested with hematemesis between 3½ and 6 years of age. (A fifth child had undiagnosed hemolytic uremic syndrome.)

A refinement of this approach was to consider polycystic kidney disease (with CHF) in infants as being different from CHF (with renal tubular ectasia) in older children [20, 64]. The infantile form (polycystic kidney disease) presents in infancy and is characterized by predominantly renal symptoms (although hepatic fibrosis can be detected by examination of the liver by microscopy). The childhood or juveile form (congenital hepatic fibrosis) presents in children and rarely in adults, and the clinical picture is dominated by the complications of portal hypertension. Renal involvement can be

diagnosed by intravenous urography (tubular ectasia), and by ultrasonography. Results of pathologic and morphometric analyses of the hepatic lesions in CHF [57, 61] added greater credence to this view, and further support came from a genetic and epidemiologic study, despite the fact that there was discordance in the age of presentation in three of these families from Finland [65].

A third approach has been to consider congenital hepatic fibrosis and ARPKD as manifestations of one gene defect expressing considerable variation within a kindred [6, 8, 53, 66–70]. It is important to note that discordance of age of onset of sibs within a kindred also had been reported by Blyth and Ockenden [5], by Lieberman et al. [20], and by Kaarainen [65]. Discordance in the age of onset (two days and one year) was noted by Chilton and Cremin [69], who concluded that there was variable expression rather than genetic heterogeneity. A family with three affected sibs was mentioned by Resnick and Vernier [68]. The propositus presented in the neonatal period, and two unaffected sibs developed portal hypertension and hematemesis, and hypetension, respectively, at later ages (juvenile form). Gang and Herrin [70] reported the features of four sibs in two families; in one, the age of onset, clinical course, and renal phenotype were concordant, and in the other they were discordant: fraternal twins (BS and AS) had different outcomes. BS died at age 20 days, while his sib was alive at 6¾ years. The ages of presentation were not noted. Discordant sibs were reported in two kindreds (patients 1,2, and 3; and 16 and 17) by Lieberman et al. [20]. In the first family, patient 1 was diagnosed at three months, the second sib at three weeks, and the third at age one day. Each had marked portal fibrosis and dilated bile ductules. In the second family, the propositus, patient 16, was examined at age six months and was found to have enlarged kidneys. Blood pressure was 150/90 mm Hg. The sib, patient 17, had sudden onset of hematemesis at age 11¼ years. These findings contradict the authors' own statement that "no instance of an infantile polycystic kidney and CHF appearing in the same family has been recorded." These authors continue: "The hepatic lesion in CHF, however, is very similar to that of infantile polycystic disease (IPCD); thus, liver biopsy is not at present adequate to distinguish CHF from IPCD. Futhermore, the course and sequelae of liver involvement in IPCD and CHF are similar". Although Kaarianen [65] noted that the clinical picture in three of 14 families with ARPKD was highly variable, she still concluded that the early lethal or *perinatal* form is a separate entity. Kaplan et al. [6] described the case of a completely asymptomatic girl, 16 years of age who came for a medical examination because her mother wanted to be certain that she did not have the same disorder from which her sib had died at birth. Her sib had died from respiratory distress at birth, and ARPKD and CHF were confirmed at postmortem examination. The patient was found to have renal tubular ectasia and CHF.

These reports support the view that ARPKD and CHF are mainfestations of the same disease [6, 53]. A physician must therefore evaluate each new sib

for evidence of ARPKD in a kindred in which one sib has CHF, and must evaluate older, apparently asymptomatic, sibs for evidence of CHF if ARPKD is diagnosed in a younger sibling.

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14. THE INHERITANCE OF NEPHRONOPHTHISIS

CLAIRE KLEINKNECHT

The term *nephronophthisis* was first used by Fanconi et al. [1] in 1951 to describe a disease characterized by its occurrence in siblings, a prominent defect in urinary concentrating ability, anemia, and progressive renal failure (in the absence of hematuria, heavy proteinuria, or hypertension) leading to death before puberty. At autopsy, the kidneys were markedly shrunken and had prominent tubulo-interstitial damage. In view of the familial occurrence with a horizontal distribution, and consanguinity in one of the two families, the authors suggested an autosomal recessive inheritance, and named the disease familial juvenile nephronophthisis (FJN). Several reports confirming the distinctive features of the disease have been published first in Europe [2–6], where 39 cases were reviewed in 1963 [6], and then in the U.S.A., where the first case was reported in 1964 [7].

During the same period, a disease characterized clinically by progressive uremia in the absence of major urinary abnormalities, except for polyuria, and characterized pathologically by the presence of medullary cysts was first reported in a child [8] and then in a few adults [9]. In 1961, Strauss [10] gathered 18 patients, most of them from the literature, who presented with renal failure and had medullary cysts. He named this condition medullary cystic disease (MCD). Habib et al. [11] were the first, in 1965, to point to the similarity between MCD and FJN. Two years later, Strauss himself [12], as well as Mongeau et al. [13], accepted the clinical and pathological identity of MCD and FJN. Other authors stressed the coexistence of medullary cysts and of

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histological lesions of FJN [14–20]. However, the MCD cases described by Strauss were rarely familial (2/18), and they occurred more frequently in adults than in children.

Although in most of the affected families the history is suggestive of a recessive inheritance, in others a dominant pattern of inheritance appears to apply. Some of these reports are based on the investigation of large kindreds, which were described as having nephronophthisis [21–23], MCD [24–27], or both [13, 14, 17]. The majority of the patients were children, but the disease was occasionally found in adults, some of them in the fourth or fifth decade of life [10, 25–28] or even older [29]. This high degree of heterogeneity made a single entity unlikely. Some authors proposed to keep the term MCD for the cases with a dominant inheritance that occur in adults and the term FJN for the juvenile recessive forms of disease. However, it must be remembered that in the past the two names have been used independently of such criteria, that a limit for age is difficult to delineate, that juvenile and adult forms coexist in the same family with either recessive [16, 30–32] or dominant [13, 17, 21, 22] inheritance, that in many instances the genetic transmission cannot be clearly defined, that the term MCD is misleading because medullary cysts exist also in FJN, and that tubulo-interstitial chronic nephritis exists in many other conditions. Thus, the diagnosis has to be based on an array of clinicopathologic findings. This is why some authors prefer to speak of the MCD–nephronophthisis complex [33, 34].

When well-defined entities, such as those resulting from an enzymatic defect, may be the result of several genetic defects, the genetic analysis of a disease with such blurred outlines and such poor diagnosis criteria as nephronophthisis is difficult to perform. It seems, however, that a major group of patients can be distinguished in whom the disease is consistent with a recessive mode of inheritance. We will first consider among them those who have the typical form of JN, then some with atypical forms, i.e., forms with unusual age at onset, forms with associated disorders and forms with an apparent dominant mode of inheritance.

NEPHRONOPHTHISIS WITH RECESSIVE MODE OF INHERITANCE

Typical juvenile nephronophthisis

Typical juvenile nephronophthisis (JN) corresponds to the disease described by Fanconi et al. [1]. The condition has long been considered as rare, but it is now generally admitted that its frequency has been underestimated and that it actually represents a major cause of end-stage renal disease (ESRD), at least among children. Nephronophthisis was the primary disease in 10% [34], 22% [35], 32% [36], and 15% (personal series) of the children treated for renal failure. Its prevalence in different countries is impossible to assess. There are only two series [34, 37], besides our own [30], that contain more than 20 patients. In addition to Europe and North America, the disease has been reported in Japan [38], South America [39], Iran [40], Israel [41], and in patients

of Arab [42], Turkish, and Indian [43] origin. In our series, many children came from North Africa. The disease has not been reported in black people.

After reviewing 101 children who presented with a chronic tubulointerstitial nephritis, we selected 93 patients who fulfilled the clinical and morphological criteria of FJN. Nonfamilial cases were included when identical to familial ones, since their exclusion would introduce a bias in the genetic analysis. Patients with an associated disorder were also included when the renal disease did not differ from that of the others, particularly from a morphological point of view.

Clinically, the disease was characterized by the appearance,in a normal child, of a defect in the ability to concentrate the urine, which resulted in polyuria, polydipsia, and growth failure, and by the absence of hematuria, hypertension (except during the terminal stage), and proteinuria (except for small amounts in the presence of severe renal failure), lack of prominent proximal tubular dysfunction, and evolution towards ESRD. The severity of anemia and acidosis paralleled the decline of GFR. Excessive sodium excretion associated with hyponatremia was evident only when renal failure was severe.

By radiology and ultrasonography, the kidneys were of normal or moderately reduced size. Ultrasonography showed the loss of corticomedullary differentiation and the presence of cysts only when the patients reached ESRD.

Examination of the renal parenchyma by light microscopy revealed a dramatic atrophy of the cortex and of the medulla and diffuse tubulointerstitial damage. The most striking feature was the extreme thickening of the tubular basement membrane, which was often multilayered and wrinkled. The thickening of the basement membrane, leading to a tire-like appearance, although not specific, is characteristic of the disease. Medullary cysts were found in only one of the 49 renal biopsies, and in all the kidneys removed at autopsy or before transplantation. The cysts could be very small and visible only by light microscopy, or very large, with almost no remaining renal parenchyma. Immunohistochemistry revealed acumulation of both type I and type II collagen in the interstitium [44]. Of possible genetic significance is the observation that staining was absent or reduced when using a particular antitubular basement membrane antibody, suggesting the presence of an abnormal basement membrane [44, 56].

The majority of reports are concordant with the description provided above [1–10, 16, 19, 30, 34–37, 45–48]. Cysts were exceptionally found on renal biopsies but more often, although not constantly [2, 3, 18, 45], on specimens taken at autopsy. There is some evidence that cysts develop late in the course of the disease and are the result rather than the cause of the atrophic process. Only in rare instances [13, 49–52] were cysts detected early by radiology, whereas they do become prominent during dialysis [53]. Lesions of tubulointerstitial chronic nephritis as found in our series were also described by others [33, 34, 44, 47, 48, 54–56].

The 93 patients in our series belonged to 74 families. In 47 cases, one or

more siblings were also affected, and in three instances first cousins had the disease. In the remaining 46 children, the disease appeared to be sporadic, probably due to the limited size of the sibship. The sex distribution among affected children was nearly equal. 142 children appeared healthy. The incidence of affected siblings, after exclusion of the probands, was 22%. The difference from the 25% expected in a recessive autosomal inherited disorder could be ascribed to late onset, the young age of some siblings, or to lack of proper investigation. In several instances, the nephropathy first appeared to be sporadic, but symptoms developed in siblings some years later. Parents were first cousins in six kindreds (8.2%) and were related in three others. This incidence of congruity is much higher than expected in the French population (0.2%) [57], but several of these kindreds came from North Africa or other countries where consanguineous matings are more frequent than they are in France. Except for one grandmother with polycystic kidney disease and one aunt with chronic glomerulonephritis, there were no instances of kidney disease in the parents or grandparents.

Taken together, these findings are consistent with a recessive autosomal inheritance. Other authors arrived at a similar conclusion based on the parents's consanguinity [1, 3, 5, 19, 37], the absence of overt renal disease in the ascendant generation, and the presence of the disease in both boys and girls. Some authors noted the high percentage of siblings reported in the literature, but concluded that familial cases were probably diagnosed and published more often than sporadic cases, resulting in a selection bias. In only one series of 21 cases did the authors calculate the percentage of affected siblings, which was 20% after exclusion of the probands, a number the authors considered as consistent with autosomal recessive inheritance [37].

Fanconi et al. [1] claimed that the age at onset and at time of death are similar within each sibship. This does not appear to be the case. In our series, the age at which the patients reached ESDR was similar in only three of 18 families, whereas it differed by more than five years in 14, and by more than 10 years in four. Such differences appear in many other reports, including those in which juvenile and adult forms were separated [31, 32].

Several pairs of twins were reported to have the disease [10, 13, 22, 36, 46]. In our series, there were two homozygous twins who had an identical clinical course. In other instances death occurred at the same age [13, 36, 46]. Children born from another marriage of one parent were unaffected [19, 33].

Some authors found a decreased urinary concentrating ability in one or two parents and in some healthy siblings, and suggested that this defect could reveal the heterozygous state [4, 7, 15]. Most authors, however, found normal kidney function, including concentrating ability, in parents and siblings of children with nephronophthisis. In our series, the concentration of the urine was measured after DDAVP administration in 13 parents and 32 siblings. In 11 parents and 23 siblings, the urine osmolality was above 800 mOsm/kg water, and they were all in apparent good health: of the remaining nine sib-

lings, seven developed nephronophthisis. This method does not allow the identification of the heterozygotes.

Herdman et al. [48] failed to detect histologic abnormalities in the kidney biopsy taken from the mother of a child with nephronophthisis. Brouhard et al. [47] found an increased thickness of the tubular basement membranes in several parts of the nephron in parents and siblings of an affected child. The results remain to be confirmed.

Nephronophthisis with unusual age at onset

Seven children in our series in whom the disease became manifest during infancy had a short survival: all reached ESRD before the age of two [58]. They had moderately enlarged kidneys, and no cysts were detected by ultrasonography. One child had cholestatic liver disease and developed portal hypertension. No liver anomaly was found in the others.

The renal morphology differed from that of typical JN by the presence of cystic dilatation of cortical tubules and Bowman's spaces and by the absence of prominent thickening of the tubular basement membranes. In the end-stage kidneys, tubules were uniformly collapsed and there were neither microcysts nor medullary cysts.

Two of these patients had a sibling who had died of uremia; none of the parents were consanguineous.

The occurrence of JN or JN-like nephropathy has been reported in only 14 additional infants [40, 59–66]. The homogeneity in regard to age at onset, clinical course, and morphologic features suggest a distinct entity. However, in many instances the picture was complicated by the presence of a heterogenous array of extrarenal abnormalities: blindness in two patients [62, 64]; cholestasis in four [59, 61, 63, 65], with increased connective tissue in the portal areas, with [65] or without [59, 64] mild bile ducts proliferation; and hepatomegaly with moderate portal fibrosis and mild duct proliferation in two [61]. Thus, it is possible that several distinct FJN-like diseases may start in infancy. Whether infants with liver or eye involvement have the same disease as older children with similar clinical manifestations is unknown. Until now, infantile and childhood forms have not been described in the same family. Of the 21 infants reported (10 boys, 11 girls), two were siblings [61], three had a sibling who died with renal failure [58, 60], and consanguinity existed in two families [40, 59]. These observations are indicative of an autosomal recessive inheritance.

Late onset is so rare among patients with autosomal recessive inheritance that it must prompt the physician to question the diagnosis. Almost all patients with a so-called *adult onset* were younger than 25 years [30–33]. Most of the older patients are likely to have a dominant form, as detailed below. Some of the sporadic cases diagnosed because of medullary cysts [9, 10, 67, 68] and histological similarity with JN are difficult to assess, although they have been included in various series such as those of Strauss [10] or Steele et al. [37].

Familial cases are exceptional; the siblings reported by Kyle et al. [28] presented with heavy proteinuria, hematuria, and prominent hypertension, suggesting another disease. Zollinger et al. [55] reported one patient aged 40 years, with eye and renal abnormalities that were also present in two of the siblings.

In conclusion, onset during the first year of life or beyond 25 years of age is most often associated with clinical, histological, or genetic features different from those of typical JN.

JN–MCD with associated disorders

Several disorders affecting various organs have been described in association with FJN–MCD-like nephropathies, with a frequency which excludes coincidence.

Eye disorders are the most frequent, either as unique [30–37, 39, 41–43, 69–88] or as one of several associated anomalies [43, 89–102]. Contreras et al. [69], Loken et al. [70], and Senior et al. [71] were the first to report three, two, and six siblings, respectively, with coarse nystagmus and blindness in early infancy who developed during childhood a renal disease leading to uremia; the morphologic pattern was identical to that of JN. In all, the diagnosis was consistent with Leber's amaurosis, the early form of hereditary retinal degeneration. In addition to visual impairment (early blindness or progressive development of visual defects beginning with night blindness), the retinal degeneration was characterized by a constant and complete extinction of the electroretinogram, preceding the development of visual and fundoscopic signs of retinitis pigmentosa [71].

An association between eye disorders and JN–MCD has been reported in more than 100 patients, the incidence being estimated to reach 40% [32]. Evidence of retinal degeneration was found in 6/63 [30], 6/27 [34], 7/21 [37], 3/10 [36], and 18/51 [88] of patients. In some of these series, the incidence may have been underestimated [30], but in others it was probably overestimated due to several reasons: 1) patients with associated disorders have been preferentially included or even specifically published; 2) the presence of eye disease has sometimes led to diagnosis; 3) the eye lesions reported were nonspecific; 4) retinitis pigmentosa may result from causes other than hereditary retinal degeneration; and 5) some families have been included in several reports.

Consanguinity was frequent and was noted in more than ten families with renal and various ocular defects [39, 41, 42, 74, 76–78, 83–87], and in sibships with additional disorders [89, 95, 100, 102]. The eye disorder was found almost exclusively in families with recessive inheritance. The exception is a patient with the dominant form of disease who had typical retinitis pigmentosa [73]. In other cases, recessive inheritance is likely despite possible renal involvement in preceding generations [91].

In our series of 93 patients, six were blind from infancy and eight had moderate to severe visual impairment at the time of investigation for renal disease. All had a flat electroretinogram. Thus, 14/93 (15%) had retinal degeneration. The percentage rises to 30% (14/46), if only children who underwent an electroretinogram were considered, but this represents an overestimation because children with visual impairment were systematically subjected to this examination. The retinopathy was always apparent before the age of 10 years.

Retinal degeneration was proven histologically [70, 87] or by electroretinogram in the minority of cases [31, 32, 36, 37, 41, 71, 74, 80–82, 84, 85, 87, 90–96]. In most reports, the presence of visual impairment or fundoscopic alterations were described only in general terms. Moreover, congenital blindness can result from other causes such as cataracts [72, 85] or coloboma [99, 101], and various types of retinitis pigmentosa have been found [31, 41]. A *subnormal* electroretinogram has been considered as an early sign of retinal degeneration, but it is now well established that only absent or extremely reduced response is diagnostic. A subnormal response was found in 22/46 of our children examined by electroretinography, but none of them developed visual defects. One of them, published as *Senior syndrome* [32, 78, 82], has normal vision 20 years later. The significance of these alterations in the retinal electrical tracing remains to be evaluated [88], particularly in patients with ESRD.

The genetic relevance of the disorders associated with JN remains unclear [31, 41, 78], particularly because of the dissociation between eye and kidney disorders within the same families [31, 37, 41, 42, 72, 74, 76–78, 83–85, 87, 91, 95]. A chance association, facilitated by consanguinity, is unlikely in view of the fact that siblings may have two, one, or none of the associated disorders [31, 41, 78]. This pattern is not compatible with a linkage between two close genes, or with a single gene causing a single biochemical defect affecting the eye and the kidney. The hypothesis of a pleiotropic gene with variable expressivity appears more reasonable [31, 41, 78].

The occurrence of dissociations within the same families, so critical to genetic interpretation, is often questionable due to the possible late onset of renal disease and, more importantly, to the lack of standard criteria for the diagnosis of hereditary retinal degeneration. In one of our families, one child had renal failure and was blind, one sibling had died at the age of six years (so that the eye disease could have passed undetected), and one sibling had no renal disease but was blind and had a coloboma [31, 78]. In another family, long considered as an example of dissociation, one blind sibling developed renal disease before the age of seven, and the other at age 24. In some cases, the retinal disorder was different from hereditary degeneration [31, 48, 77, 85], as indicated by the presence of abnormal but not flat electroretinograms [31, 77, 85]. In the report of Meier and Hess [74], three siblings had the typical association of kidney disease and retinal degeneration, one had only myopia, and

one had questionable renal involvement. In many instances, siblings with no renal impairment had abnormal retinal electric tracings [42, 76, 84] but no visual defects. Finally, in the report of Steele et al. [37] of six families with ocular and renal disorders, a dissociation was noted only in one. It must also be reminded that retinitis pigmentosa may be encountered in a variety of conditions that also affect the kidney [43]. We have found retinal degeneration in patients with renal dysplasia, Jeune's syndrome, tubulo-interstitial nephritis morphologically different from JN, and in some of the infantile microcystic forms of renal disease described above [62, 64]. Thus, the detection of the ocular lesion has no diagnostic value.

Disorders of the central nervous system may also be present in JN. The most common are mental retardation and cerebellar dysfunction. Mental retardation was present in one of the families reported by Fanconi et al. [1], in four of our 93 patients, and in about 15 other reported families [5, 35, 37, 46, 69, 70, 75, 76, 85, 89, 90, 93, 96, 99, 101, 102, 104]; it was often associated with eye lesions [69, 70, 75, 89, 93, 96, 99, 101]. Cerebellar ataxia was observed in two siblings with retinal degeneration in our series, and has been reported to occur in other patients, with [35, 37, 90, 93, 104] or without [46, 92, 94, 101] mental retardation, in all but one instance [104] in association with retinal abnormalities.

Liver involvement was first described by Boichis et al. [105] who observed a consanguineous family with three uremic children who had renal lesions consistent with JN and hepatosplenomegaly. The liver function tests were normal. Biopsies revealed portal fibrosis without bile duct proliferation. Two additional siblings were probably affected. Subsequently, several investigators reported hepatic fibrosis and JN, particularly in infants. Most of the articles are listed in table 14–1. Additional cases are those of Waldherr et al. [34] and Gomez-Campdera [35]. All had hepatomegaly and moderate portal fibrosis with mild bile duct proliferation, and hence a pattern different from that of classical congenital hepatic fibrosis, where biliary dysgenesis is prominent. We observed only one case who had cholestasis, and Steele et al. [37] found none, even at autopsy.

Bone abnormalities were first described by Mainzer et al. [92] in siblings presenting with retinal degeneration and cerebellar ataxia. They have also been observed by other authors [43, 94, 97, 100, 106], always in association with other disorders: retinal degeneration in all but two [43, 106], hepatic fibrosis in all but two sibships [92, 97], and cerebellar ataxia in two [92, 94]. Two of these children were diagnosed later as having Jeune's syndrome [43].

Other associations have been observed occasionally and may be coincidental, except for hyperhydroxyprolinuria [31, 89, 95], which may result from hyperparathyroidism, and gout, which was noted only in patients with dominant inheritance. Chromosomal abnormalities were found by Sarles et al. [91] but in most other instances the karyotype was normal.

Table 14-1 lists the patients who had at least three organs affected, includ-

Table 14-1. Multiple organ involvement in juvenile nephronophthisis

		Affected organs				
		Eye	Liver	Brain		
Author	Year			Mental retardation	Cerebellar ataxia	Bone
Debakan [90]	1969	RD (flat ERG)	HF	+	+	−
Fontaine [93]	1970	RD (autopsy)	Cholestasis (no HF)	+	−	−
Mainzer [92]	1970	RD (ERG)	—	−	+	+
Proesmans [96]	1975	RD (flat ERG)	HM—HF	+	−	−
Popovic [94]	1976	RD (flat ERG)	—		+	+
Robins [106]	1976	—	HM—HF	−	−	+
Freycon [103]	1977	—	HM—HF	+	−	−
Diekman [97]	1977	RD	—	+	+	−
Weber [99]	1978	Coloboma	HM—HF	+	+	−
Delaney* [98]	1978	Ret. hypoplasia	HM—HF	+	−	−
Dietrich [64]	1980	Coloboma	HM—HF	+	+	−
Bodaghi [100]	1980	R. pigmentosa	HM—HF	−	−	+
Donaldson [43]	1985	?	HM—HF	−	−	+
"	"**	RD	—	−	+	−

*Twins.
**In addition to mitochondrial cytopathy.
RD: Retinal degeneration; ERG: Electroretinogram; HF: Hepatic fibrosis; HM: Hepatomegaly.
Bone abnormalities were mainly cone-shaped epiphyses.

ing the kidneys. There is an obvious heterogeneity, regarding particularly the eye disorder and the occurrence of bone disease. Obviously, these cases are not likely to belong to the same genetic entity. Two cases [43, 106] are probably Jeune's syndrome [43]. Whether the renal lesions are actually identical to those of JN is difficult to determine, but seems doubtful. As previously stated, of three patients we observed with eye, liver, and bone disorders, one had renal dysplasia and the two others were considered as differing from JN, despite the presence of medullary cysts in one. Other authors have stressed the possible occurrence of tubulo-interstitial and/or renal cysts in several syndromes such as Jeune's [107], Moon–Bardet–Biedl, and Ellis van Crevel, in which retinal, hepatic, or bone lesions may be found [108, 109].

Thus, the existence of associated disorders must not be considered as evidence of JN, but should rather elicit investigation for more specific features of the disease.

NEPHRONOPHTHISIS–MEDULLARY CYSTIC DISEASE WITH DOMINANT INHERITANCE

The occurrence of this disease in succeeding generations suggests a dominant inheritance. Such a genetic pattern of inheritance was assumed in only 29 families [13, 14, 17, 20–27, 28, 33, 73, 110–112], and in half of them is questionable.

Goldman et al. [24] were the first to report a large kindred with dominant inheritance. Renal failure and medullary cystic disease were recognized in the

first four of the five generations. Eighteen of the 25 members of the first three generations either manifested overt renal failure or transmitted the disease to their offspring. Males and females were affected with similar frequency. No consanguinity was observed in this kindred. A follow-up disclosed the disease in the fifth generation [25].

Unfortunately, most of the subsequent reports include only one family. An exception is the report of Grateau et al., which includes three families [32]. Victorin et al. [21] described nine affected children in families; however, only six had overt renal disease, and of the four whose kidneys were examined, only one had medullary cysts. In nine of the families in which the trait was considered to be dominant, the diagnosis was based on the detection of medullary cysts by radiology or ultrasonography in at least two generations [13] or on the histology of the renal parenchyma [19, 21, 24, 25, 27, 32, 33, 112]. In the remaining families, the diagnosis was based on history. Half-brothers were affected in some families [23, 26, 112], and consanguinity was found in none. Some authors have raised the possibility of an X-linked inheritance [24], but transmission from father to son was observed in many families [24–26, 32].

In the family reported by Giangiocomo et al. [17], in one of the families reported by Mongeau et al. [13], and in two of the eight families reported by Victorin et al. [21], both parents were found to have normal renal function, including urinary concentrating ability, while members of the preceding generation had renal disease. This findings raises the possibility of variable penetrance. A decreased maximal urinary concentration or glomerular filtration rate in only one parent has also been considered as suggestive of a dominant inheritance with incomplete penetrance [15].

Age at onset and the age at which patients reach renal disease appears to be different in dominant and recessive forms of JN. Most of the patients reported to have dominant inheritance were adults, the great majority over 30 years of age [29]. In the six children reported by Giangiacomo et al. [17], the diagnosis of MCD was unproven or questionable in the previous generation. Among adults, ESRD was found to be reached at a mean age of 47 years in the dominant form and at 30 years in the recessive form of the disease. In the family described by Burke et al. [20], the age at which the patients required dialysis ranged from seven to 48 years. Moreover, most of the children considered to have dominant inheritance differed from those with JN in regard to the clinical findings. The patients of Mongeau et al. [13] had large cysts detected by radiology coexisting with moderate renal failure; the twins reported by Makker [22] had normal urinary concentrating ability, hematuria, and heavy proteinuria; and the family of Burke et al. [20] had hyperuricemia and gout, all signs never found in JN. Polyuria seemed much less severe in patients with dominant inheritance than in JN. Hypertension, which is exceptional in JN, was frequent, severe, and often associated with vascular lesions in the dominant form of the disease [21, 26, 110, 112]. Finally, the detection of large cysts by radiology before the terminal stage of the disease was frequent in patients with dominant

inheritance [13, 26, 27, 29, 32], whereas it was exceptional in JN. Whether the high incidence of hypertension and large cysts is related to older age and longer duration of the disease, or indicates a different pathologic process, cannot be determined.

Thickening of the tubular basement membrane is so characteristic of JN that we considered it necessary for diagnosis. This feature was only rarely mentioned among patients with dominant inheritance. Grateau et al. [32] observed basement membrane thickening in one of six such patients. Victorin et al. [21] described a "mild thickening" of basement membrane in parents, contrasting with the "considerable" thickening observed in children. The lesion was also noted by Collan et al. [23] and Avasthi et al. [33].

The numerous disorders associated with JN have not been detected in patients with dominant inheritance. In one case in which chronic tubulointerstitial nephritis coexisted with retinitis pigmentosa and bone anomaly [73], the bone disease differed from that observed in FJN, and the electroretinogram excluded retinal degeneration. In another family, the patients had retinitis pigmentosa, and the history of uremia in a great-grandmother and her sister suggested a dominant inheritance. However, the parents were normal [91].

The only disorder consistently associated with dominant tubulo-interstitial nephritis with [20] or without [111] medullary cysts is hyperuricemia. In our series, the only instance of parent to child transmission was that of tubulo-interstitial nephropathy and gout. It is likely that this association represents a distinct entity [113, 114]. It should be remembered that in young patients renal insufficiency due to uric acid deposition can occur in the absence of attacks of gout [113, 114].

In summary, diseases associated with medullary cysts or tubulointerstitial nephritis resembling nephronophthisis may have a dominant inheritance. Such cases are rare, however, and most of them seem to have a phenotype different from that of JN that may correspond to several ill-defined conditions. JN, on the other hand, represents a disease entity occurring in childhood or early adulthood, with a recessive autosomal mode of inheritance. However, there is no pathognomonic feature so that the diagnosis is only more or less probable, never certain. The presence of medullary cysts is not diagnostic, because they may be encountered in other conditions, including in patients treated by dialysis. Moreover, they may develop late during the course of typical JN. Thickening of the tubular basement membranes provides a high degree of suspicion, but its diagnostic value remains to be proven. Finally, whether even the most typical cases are due to one or several gene defects remains unknown.

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15. GENETICS OF UROLITHIASIS

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Urolithiasis is a major worldwide health problem that primarily afflicts adults, although children are not immune from the disorder. In the United States, nearly 15% of adults suffer from urinary stones; in other geographic regions (i.e., Southeast Asia), urinary calculi are endemic and the incidence is even greater. In some patients, environmental factors, such as diet, climate, composition of drinking water, or fluid intake, may be responsible for the formation of urinary stones. On the other hand, a genetic basis of urolithiasis is present in many patients in whom no exogenous explanation for calculus formation is found. Certainly, a genetic basis for specific metabolic disorders that lead to urolithiasis, such as cystinuria and oxaluria, is well established. Few comprehensive reviews of the genetic basis of urolithiasis are available.

In order to discuss the genetic basis of urolithiasis, one must consider the urinary environment that conspires to allow the formation of a stone. Urinary stones may result from either a reduction in natural urinary inhibitors of crystallization or from excessive excretion of lithogenic salts. Table 15-1 lists etiologies of urolithiasis in adults and children from single center studies [1, 2]. The following discussion will address the current understanding of the genetic basis of many of these disorders. Oxaluria will be reviewed in chapter 16.

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Table 15-1. Etiologies of urolithiasis in adults and children in the United States [1, 2]

	Adults $n = 216$		Children $n = 112$	
	n	%	*n*	%
Idiopathic	26	12	21	18.8
Hypercalciuria	139	64	47	42.0
(Absorptive)	(123)		(18)	
(Renal)	(10)		(29)	
Uric acid	5	2	4	3.6
Hyperuricosuric calcium oxalate	21	10	—	—
Hyperparathyroidism	14	7	—	—
Cystinuria	0	0	5	4.5
Oxaluria	5	2	3	2.7
Infection	5	2	15	13.4
Other	1	1	17	15

IDIOPATHIC CALCIUM UROLITHIASIS

In many patients with urolithiasis (usually composed of calcium oxalate), no etiology is ascertained even after extensive evaluation. A family history is extremely common in such patients. The percentage of idiopathic adult stone formers in whom urolithiasis also afflicts first-degree relatives ranges from 13% to 60% (table 15-2). In all studies, urinary stones occurred with greater frequency in fathers (9%–15%) and brothers (14.2%–20.5%) of stone patients than in mothers (1.7%–11%) or sisters (2.2%–8.2%) [5, 6]. Resnick determined that 48% of male siblings of stone patients would develop urolithiasis if they lived to the age of 75 years [5]. Such patient populations have suggested a polygenic form of inheritance [5, 6], although an autosomal dominant transmission with clinical expression being modified by sex cannot be excluded. Environmental factors may also be important in determining clinical expression, since wives of men who form urinary calculi have a greater risk of urolithiasis than do wives of men without urolithiasis [6]. In 23 children with idiopathic urolithiasis at our center, 9 (39%) have a sibling, parent, or grandparent with urolithiasis (Stapleton, Miller, Noe, 1988, unpublished data). In both children and adults, urinary stones are more common in males than in females and are rare in black patients. One potential explanation for the male predilection for calculus formation may be the lower urinary citrate excretion in males than in girls and women [9, 10]. Similarly, urinary calcium excretion is lower in black adults [11] (but not in black children [12], than in white control subjects; further clarification of the factors responsible for race and gender differences in stone occurrence is needed.

Due to the absence of an understanding of the etiology of calculus formation, it is impossible to determine a gene product that might allow exploration of a specific gene defect. In fact, it is probable that this group represents a heterogeneous population of patients. Clinical surveys have examined whether

Table 15-2. Family history of urolithiasis in patients with idopathic urinary calculi

Reference	Patients with urolithiasis (*n*)	% with positive family history
3	77	13
4	300	20
5	106	13.9
6	377	29.6
7	50	60
8	380	55.4

urinary calculi could be linked to ABO phenotypes [13] or HLA types [14]. Clauderella and associates found that ABO phenotypes in a population of patients with urolithiasis did not differ from the general population [13]. A weakly significant difference in urinary calcium excretion was observed between blood groups A and O ($p < 0.05$), with hypercalciuria (urinary calcium excretion >300 mg/day) occuring more often in 16 patients with blood group A. HLA frequencies (range 0.06–0.68) in patients with idiopathic calcium urolithiasis failed to demonstrate an association with any specific HLA allele [14].

A more promising line of investigation was reported by Baggio and coworkers who examined oxalate transport across erythrocyte membranes in 98 patients with idiopathic and recurrent calcium urolithiasis [15]. Erythrocyte oxalate flux was $-1.10 \pm 0.95 \times 10^{-2} \text{min}^{-1}$ in patients with idiopathic urolithiasis compared to $-0.31 \pm 0.12 \times 10^{-2} \text{ min}^{-1}$ in control subjects ($t = 4.14$, $p < 0.0001$). Seventy-eight of the 98 patients with urolithiasis demonstrated oxalate transport in excess of +2SD of controls. Increased erythrocyte oxalate flux was observed in successive generations in four families. Abnormal oxalate flux was distributed equally between males and females. This transport abnormality was consistent with an autosomal dominant mode of inheritance. Unfortunately, the relationship of this erythrocyte oxalate defect and urinary stone formation is unclear; urinary oxalate excretion did not correlate with erythrocyte oxalate transport. Interestingly, the diuretics, hydrochlorothiazide and amiloride (both known to reduce calculus formation), corrected erythrocyte oxalate flux to values within the normal range in patients with urolithiasis. Male siblings of patients with increased erythrocyte oxalate flux had hyperoxaluria after an oral [^{14}C] oxalate oral tolerance test.

The presence of abnormal erythrocyte oxalate transport in 70% of adults with idiopathic urolithiasis makes this study the most promising marker for stone formation heretofore reported, although this study has not yet been confirmed in other populations of patients with urolithiasis. The membrane defect responsible for this abnormality in oxalate transport is still unproven. A specific anion transport protein–band 3 protein–is a component of erythocyte membranes [16]. Inhibitors of band 3 protein transport reduce erythrocyte oxalate flux to normal values in patients with a history of urolithiasis [16].

Studies of band 3 protein may therefore lead to the identification of a gene product responsible for this transport defect [16].

Therapy for idiopathic urinary calculi is limited to modification of environmental factors that participate in stone formation. Clarification of the genetic bases of idiopathic urolithiasis may allow specific diagnostic grouping of many such patients and ultimately more specific therapies.

HYPERCALCIURIA

Increased urinary calcium excretion is the specific metabolic abnormality most frequently associated with urolithiasis [1, 2]. Approximately 40% of both children [2] and adults [1] with urinary calculi excrete greater than 4 mg (0.1 mmole)/kg/day in their urine while ingesting an unrestricted diet. Urinary calcium excretion in black adults appears to be less than in white adults [11], and upper normal values for blacks have not been determined. In children, urinary calcium excretion is similar in boys and girls and does not differ between black and white subjects [12]. Urinary calcium excretion relative to body weight does not systematically vary with age; however, urinary calcium excretion is greater during early puberty than later in adolescence [12]. Calcium excretion is influenced by a large number of physiological, hormonal, and pharmacological factors. This discussion will focus on idiopathic hypercalciuria, renal tubular acidosis, and hyperparathyroidism.

Idiopathic hypercalciuria

Increased urinary excretion of calcium may result from either an increased filtered load of calcium or defective renal tubular reabsorption of calcium [17]. An increased filtered load may result from excessive bone resorption (i.e., hyperparathyroidism) or from primary hyperabsorption of calcium by the gut (absorptive hypercalciuria). Absorptive hypercalciuria is the most common type of idiopathic hypercalciuria [1]. The pathogenesis of increased gastrointestinal calcium absorption may be the result of excessive production of 1,25$(OH)_2$ vitamin D, perhaps due to a renal tubular phosphorus leak [18, 19]. Not all patients with absorptive hypercalciuria have increased serum concerntration of 1,25$(OH)_2$ vitamin D, however. In absorptive hypercalciuria, serum concentrations of parathyroid hormone should be normal or decreased [17].

Renal hypercalciuria is believed to result from an isolated defect in renal tubular reabsorption of calcium with secondarily increased serum concentration of parathyroid hormone [17, 20]. A more generalized tubular transport defect has been reported in some patients with renal hypercalciuria [21]. The separation of idiopathic hypercalciuria into two distinct disease entities has been supported by sound physiological reasoning [17] and by urinary calcium excretion during an oral calcium loading test [17, 22]. Recently, the categorization of idiopathic hypercalciuria has been challenged because some patients with absorptive hypercalciuria also have a defect in renal tubular calcium transport [23], because the oral calcium loading test has been found to

be unreliable in many instances [24–26], and because many individuals with renal hypercalciuria (as diagnosed with the oral calcium loading test) have normal serum concentrations of parthyroid hormone [22].

An autosomal dominant pattern of genetic transmission for both renal and absorptive hypercalciuria has been suggested by Pak [17]. As many as 82% of patients with hypercalciuria have a history of a family member with urolithiasis [24]. Coe and associates examined 73 relatives of 9 patients with hypercalciuria and urolithiasis [25]. Forty-three percent of the first-degree relatives had urolithiasis; stones occurred with equal frequency in male and female relatives. In addition, 11 of 24 siblings had hypercalciuria and 7 of 16 progeny had hypercalciuria. In each family in whom both parents of the index case were studied, at least one parent also had hypercalciuria. Unfortunately, urolithiasis and hypercalciuria were not necessarily related in these pedigrees [25]. In 24 children with hypercalciuria and urolithiasis, Szelid found that 13 of 15 patients with familial hypercalciuria had renal hypercalciuria [26]; in contrast, absorptive hypercalciuria was diagnosed in 8 of 9 children with nonfamilial urolithiasis and hypercalciuria. Finally, Hymes and Warshaw examined calcium–phosphorus metabolism in 29 relatives of six children with hypercalciuria [27]. These authors observed somewhat higher serum concentrations of $1,25(OH)_2$ vitamin D and greater urinary calcium excretion in 14 relatives of children with hypercalciuria. These studies suggest an inherited role of $1,25(OH)_2$ vitamin D in the pathogenesis of hypercalciuria; however, the data fail to conclusively demonstrate that the hypercalciuric subjects are a homogeneous group of patients.

Family studies of patients with absorptive hypercalciuria have suggested an autosomal dominant pattern of inheritance [28, 29]. Pak reported absorptive hypercalciuria associated with urolithiasis in six individuals in two generations [29]. Weinberger also described four families in whom absorptive hypercalciuria was apparently inherited as an autosomal dominant gene [28]. A fascinating pedigree with hereditary absorptive hypercalciuria and renal tubular acidosis has been described by Hamed and associates [30]. Eighty individuals in five generations were studied. Distal renal tubular acidosis (both complete and incomplete) was observed in eight subjects in the first three generations. Absorptive hypercalciuria was identified in nine subjects in the second through the fourth generations. In the younger individuals, hypercalciuria was not associated with renal tubular acidosis. The authors interpreted their findings to represent an autosomal dominant gene defect in which absorptive hypercalciuria was the primary abnormality and renal tubular acidosis resulted from nephrocalcinosis associated with hypercalciuria [30]. Another large kindred in which hypercalciuria preceded the onset of distal renal tubular acidosis has been reported [31]. In this kindred six individuals had nephrocalcinosis and four had renal tubular acidosis; 13 had hypercalciuria without renal tubular acidosis. These two families provide strong suggestive evidence that prolonged hypercalciuria may injure the renal tubule and result in a renal acidification defect.

Table 15-3. Comparison of absorptive and renal hypercalciuria

Type of Hypercalciuria	Absorptive	Renal
Produces urolithiasis	Yes	Yes
Causes hematuria in children	Yes	Yes
White race predominates	Yes	Yes
Fasting urine calcium	Normal	↑
Serum parathyroid hormone	Normal or ↓	↑ or normal
Urinary cAMP	Normal	↑ or normal
Bone mineralization	Normal	↓ (Adults)
Proposed genetic transmission	Autosomal dominant	Autosomal dominant

Recently, extensive studies of 59 closely related member of a Bedouin tribe suggested a relationship between idiopathic hypercalciuria and a newly described condition, hereditary hypophosphatemic rickets with hypercalciuria [32]. It was hypothesized that a renal tubular phosphorus leak led to increased serum concentrations of $1,25(OH)_2$ vitamin D and thus hypercalciuria. The genetic transmission of these conditions could represent an autosomal recessive inheritance with two gene defects or an autosomal dominant inheritance with variable expressivity [32].

Large kindreds with renal hypercalciuria have not been reported. Pak reported that 38.5% of patients with renal hypercalciuria have a family history of urolithiasis [12]. Among 31 children with renal hypercalciuria in our center, 22 (71%) had a family history of urolithiasis. Among relatives of a group of 22 children with urolithiasis and elevated fasting urinary calcium excretion, urinary excretion of calcium, phosphorus, sodium, and potassium was greater than in family members of a control group [21]. Specific pedigree information was not provided. These authors suggest that their patients with hypercalciuria were a group of individuals who represented the upper range of normal values rather than a metabolic variant. Features of absorptive and renal hypercalciuria are summarized in table 15-3.

Spontaneous hypercalciuria has been described in Sprague–Dawley rats [34]. Mating of hypercalciuric rats produces progeny in whom urinary calcium excretion is also excessive. Initial studies suggested that hypercalciuria was due to a renal tubular defect in sodium and calcium transport [34]; however, more recent studies are consistent with an increased gastrointestinal transport of calcium without increase in serum concentrations of $1,25(OH)_2$ vitamin D [35]. It is possible that rats with genetic hypercalciuria may have more than one genotype.

A number of syndromes associated with hypercalciuria have been reported [33, 36–41]. Gentil [36] and Royer [37] described children in whom hypercalciuria was associated with growth failure, rickets, osteomalacia, nephrocalcinosis, and renal tubular dysfunction. These syndromes are distinct from vitamin-D-deficient, resistant, or dependent rickets. The pattern of inheritance in some families is obscure; however, in some families, an autosomal recessive

pattern is suggested [33]. Parental consanguinity has been noted in three families [37–39]. The marked bone abnormalities clearly distinguish this group of children from patients with idiopathic hypercalciuria.

Renal tubular acidosis

Nephrocalcinosis and nephrolithiasis are important complications of type I (distal) renal tubular acidosis [42]. The pathogenesis of stone formation in type I renal tubular acidosis may be related to 1) the constantly alkaline urinary pH, which promotes the precipitation of calcium phosphate salts, 2) increased urinary calcium excretion from bone resorption, and 3) decreased urinary citrate (an inhibitor of nucleation). The relative contribution of each of these factors has not been determind. Type II (proximal) renal tubular acidosis is not associated with urolithiasis and none of the three stone-promoting risk factors in type I (distal) renal tubular acidosis is present in the type II (proximal) form of this condition.

Two families (vide supra) in whom chronic hypercalciuria has appeared to lead to a distal renal tubular defect in maintaining a H+ gradient have been reported [30, 31]. In other families, type I renal tubular acidosis is the primary inherited renal tubular defect. The genetic basis for renal tubular acidosis was reviewed extensively by Seldin and Wilson [43]. In some families, type I renal tubular acidosis clearly has been inherited as a Mendelian dominant trait [44–47]. Within these pedigrees, occasional individuals suffered from nephrolithiasis prior to developing renal tubular acidosis [45]; however, in all pedigrees, most affected individuals did not have nephrocalcinosis. The clinical expression of this autosomal dominant genetic trait may include nephrocalcinosis, urolithiasis, or only metabolic acidosis. In some families, in whom only multiple members of a single generation demonstrate type I renal tubular acidosis, an autosomal recessive trait is suggested [43, 48]. In one such family, type I renal tubular acidosis was associated with nerve deafness, and nephrocalcinosis was observed in these brothers [54]. Most cases of distal renal tubular acidosis are sporadic. In the families with inherited renal tubular acidosis, no specific gene defect or gene locus has been identified. Future genetic studies are needed to determine a gene product so that appropriate genetic probes can be developed.

Hyperparathyroidism

Hyperparathyroidism is an unusual cause of urolithiasis in adults and a rarity in children with urinary calculi. In hyperparathyroidism, excessive urinary calcium excretion is usually, but not always, associated with hypercalcemia. A familial occurrence has been documented in some families with parathyroid adenomas [49–51]. The parathyroid tumors are benign and multifocal, and present in the adult years with symptoms of hyperparathyroidism. The most likely mode of inheritance is an autosomal dominant trait. The gene that controls PTH synthesis is located on the 11th chromosome [52]. No specific

gene defect in autosomal dominant parathyroid adenomas has been identified. Familial hyperparathyroidism has also been associated with chief-cell hyperplasia [53]. It is unclear whether this autosomal dominant condition is truly distinct from parathyroid adenomas.

Hyperparathyroidism is a feature of multiple endocrine neoplasia (MEN). This syndrome of multiple hyperfunctioning endocrine tumors is inherited as an autosomal dominant trait. Hyperparathyroidism is the most common endocrine neoplasia found in patients with the syndrome. Other endocrine tumors include adrenal, thyroid, and pituitary chromophobe adenomas, as well as carcinoid tumors [55]. Hyperinsulinemia and increased secretion of other pancreatic peptides are also found. Two types of mutliple endocrine neoplasia have been described. Type I MEN is characterized by hyperparathyroidism in 95% of patients and frequently by hypercalciuria. Excessive secretion of gastrin is also common. The gene locus for type I MEN has been mapped to the long arm of chromosome 11 and is tightly linked to the muscle phophorylase gene [107]. In type II MEN, hyperparathyroidism occurs in 20%–30% of affected patients. Furthermore, type II MEN has been further subdivided into types IIa and IIb. Medullary carcinoma of the thyroid frequently is found in patients with type IIa. The locus for MEN type IIa has been assigned to chromosome 10 and is linked to the interstitial retinol-binding protein gene [108]. Type IIb MEN has a low incidence of hyperparathyroidism but is typified by a Marfan-like body habitus, mucosa neuromas, and ganglioneuromas [56].

Evaluation and therapy of patients with hypercalciuria and urolithiasis

Documentation of excessive calcium excretion is established when urinary calcium excretion exceeds 4 mg/kg/day while the patient is ingesting a routine diet. When hypercalciuria is identified, serum concentrations of parathyroid hormone, calcium, phosphorus, bicarbonate, and potassium should be assayed to exclude hyperparathyroidism, renal tubular acidosis, hypercalcemia, and generalized renal tubular disorders. To further characterize the type of idiopathic hypercalciuria, 24-hour urinary calcium excretion should be reexamined after a week of dietary calcium and sodium restriction. In some instances, an oral calcium loading test should be performed [21]. When family members have a history of urolithiasis or hematuria, similar laboratory investigations should be undertaken in affected relatives. We routinely obtain serum bicarbonate concentration and 24-hour urinary citrate excretion to screen for type I renal tubular acidosis and to determine if citrate therapy might be warranted.

Therapy for renal hypercalciuria includes hydrochlorothiazide, a low sodium and oxalate diet, and a high fluid intake. Patients with absorptive hypercalciuria should ingest a diet restricted in oxalate, sodium, and calcium, and high in fluids. Alkali therapy should be given to patients with renal tubular acidosis. Surgical extirpation is indicated for primary hyperparathyroidism.

Table 15-4. Solubility of purines in body fluids [60]

	pH	Uric acid (mg/dl)	Xanthine (mg/dl)	Hypoxanthine (me/dl)
Serum	7.4	7	10	115
Urine	5.0	15	5	140
Urine	7.0	200	13	150

Citrate therapy should be given to patients with reduced urinary citrate excretion.

Uric Acid Urolithiasis

Uric acid is a weak organic acid with a pKa of 5.75 for the ionization of hydrogen atoms at position 9 and a pKa of 10.3 for the hydrogen atom at position 3. The ionized forms of uric acid form salts such as mono- or disodium or potassium urate. At physiological pH, uric acid exists almost entirely as monosodium urate. The solubility of monosodium urate is nearly 15 times that of uric acid in aqueous solutions. In human plasma, the saturation concentration of monosodium urate is approximately 7 mg/dl.

Uric acid and precursors of uric acid are poorly soluble. The saturation of uric acid and xanthine is shown in table 15-4. Urinary pH not only influences the relative concentration of monosodium urate but also the solubility of urate. Thus in an acid urine (less than pH 5.5), uric acid will predominate and will have minimal solubility. In addition to pH, urinary urate solubility will be decreased by sodium and lower temperature but increased by potassium, lithium, and urea [57].

The metabolic pathways leading to uric acid are shown in figure 15-1. Normal concentrations of uric acid in serum are perilously close to the limits of solubility. Normal values for serum uric acid concentration range from 3.5 to 7.9 mg/dl in men and from 3 to 6 mg/dl in women [58], and are lower in healthy children [59]. Increased concentration of uric acid in the serum is due to either increased production of uric acid or decreased elimination. Most genetic studies of hyperuricemia have primarily examined families in whom hyperuricemia is associated with excessive urinary excretion of uric acid, thereby indicating excessive production. Two families with hyperuricemia and reduced urinary uric acid excretion have been carefully examined [61]. In these two families, increased renal tubular reabsorption of uric acid appeared to be inherited as an autosomal dominant trait. Men were affected more frequently than women (men = 26 of 29 individuals at risk). Such patients are at low risk for urinary stones due to the low urate excretion.

Approximately 75% of the daily urate excretion is eliminated by the kidneys. Excessive excretion of uric acid is considered to be greater than 700 mg per day in men and 600 mg per day in females [62]. Normal values for urinary uric acid excretion vary with age but are constantly less than 0.6 mg/dl

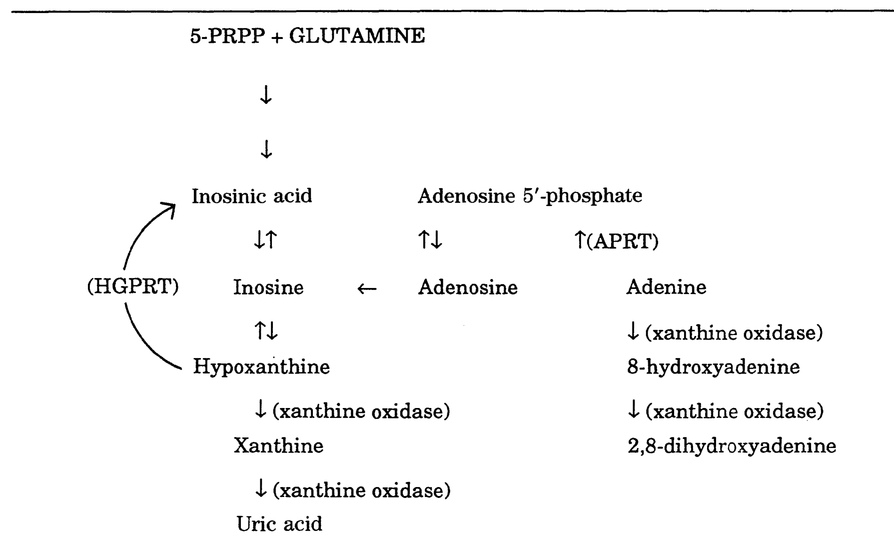

Figure 15-1. Metabolic pathways involved in purine disorders leading to urolithiasis. APRT = Adenine phosphoribosyltransferase.

creatinine clearance [63]. Renal clearance of uric acid is determined by glomerular filtration, tubular reabsorption, tubular secretion, and postsecretory reabsorption [64]. The fractional excretion of uric acid is less than 12% in adults but is considerably higher in children [59]. Increased excretion of uric acid is associated with an increased risk for both uric acid and calcium oxalate urolithiasis [65]. In patients with gout, the prevalence of urinary stones is 35% when urinary uric acid excretion is between 700–1000 mg per day and is 50% when uric acid excretion exceeds 1000 mg per day [66].

Idiopathic hyperuricemia

Since antiquity, gout has been recognized to be familial. In the United States, nearly 40% of patients with symptomatic hyperuricemia have a relative with gout [67], and asymptomatic hyperuricemia has been identified in as many as 27% of relatives of patients with gout [68]. Talbot found that 34 of 136 asymptomatic relatives of patients with gout had serum concentrations of uric acid in excess of 6.0 mg/dl, and 80% of hyperuricemic relatives were male [68]. Symth and associates studied 19 families with hyperuricemia and concluded that the genetic defect was transmitted as an autosomal dominant trait [69]. Similarly, Stecher et al. evaluated 201 members of 44 families with gout and concluded that hyperuricemia was inherited as an autosomal dominant gene in some families, as an autosomal recessive defect in some families, or as an autosomal dominant gene with low penetrance in some families [70]. Hauge and Harvald concluded that familial hyperuricemia could best be explained by multifactorial inheritance [71]. A similar conclusion was reached by

Neel and associates [73] who evaluated families initially examined by Symth et al. [69] and rejected the previous designation of autosomal dominant inheritance and suggested, instead, a multifactorial inheritance. The gene(s) responsible for hyperuricemia has not been identified.

In patients with idiopathic hyperuricemia within the United States, the prevalence of uric acid stones ranges from 5%–33% [72]. In a review of 1258 patients with gout, Yu and Gutman reported urolithiasis in 22% [66]. Thus, the risk for uric acid urolithiasis is approximately 100 times the risk for population of healthy, normouricemic adults [62]. In Israel, 75% of gouty subjects are afflicted with stones [73]. The risk of stone formation in patients with gout is related to the severity of hyperuricemia. In men, the prevalence of uric acid stones increases from 12.7% at a serum concentration of 7–8 mg/dl to 40% when the serum urate concentration exceeds 9.0 mg/dl [74]. Over 50% of patients with serum uric acid concentration greater than 13.0 mg/dl have urolithiasis [66]. In 40% of patients with gout, urolithiasis precedes gouty arthritis symptoms. In 84% of patients with gout, stones are exclusively made of uric acid; calcium oxalate was a primary or secondary component in 12%, and calcium phosphate in 4% [74].

In some patients with uric nephrolithiasis, the serum uric acid concentration is normal. A familial form of uric acid lithiasis associated with constantly acid urine and increased excretion of titratable acid has been reported [75]. The mechanism of high urinary acid excretion and the formation of urate stones is unknown in such patients. It appears that this disorder is inherited as an autosomal dominant trait with high penetrance in both sexes.

DEFICIENCY OF HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HGPRT)—LESCH—NYHAN SYNDROME

Complete or nearly total absence of HGPRT is characterized by a constellation of symptoms that includes choreoathetosis, spasticity, mental retardation, and self-mutilating behavior [76]. All patients are male. HGPRT catalizes the conversion of hypoxanthine to inosinic acid and guanine to guanylic acid in the presence of phosphoribosylpyrophosphate (figure 15-1). In classic Lesch–Nyhan syndrome, HGPRT activity ranges from 0% to 5% [77]. The diagnosis is made by assaying enzyme activity in erythrocytes. Partial deficiency in HGPRT has also been reported [78]. HGPRT enzyme activity is high in the brain, particularly in the basal ganglia [79].

HGPRT is coded by a single gene that is located distally in the long arm of the Y chromosome between the genes for PP-ribose-P synthetase and glucose-6-phosphate dehydrogenase [80, 81]. The structural gene for HGPRT has an estaimated length of 34 Kb [81]. The genetic control of HGPRT has been studied extensively due to the expression of the enzyme in many tissues and because the structures of normal HGPRT and mRNA have been defined [81]. Purification of mutant HGPRT genes in patients with parital or complete deficiency of HGPRT has led to the identification of single amino acid sub-

stitutions in five known variants of HGPRT. In a patient with Lesch–Nyhan, a substitution of asparagine for arginine at position 193 of the HGPRT has been identified [81].

The lack of feedback control by the purine salvage pathways leads to massive overproduction of uric acid. Uric acid excretion is dramatically increased [63]. Uric acid stones are extremely common. Uric acid crystalluria is uniformly present in untreated patients. Aggressive management with allopurinol and urinary alkalinization is essential. Due to the massive overproduction of uric acid, allopurinol will lead to dramatic increases in xanthine excretion and may lead to xanthine stones [82]. Repeated episodes of urolithiasis may lead to renal failure in some patients.

DEFICIENCY OF ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT)

Like HGPRT, APRT is a purine salvage enzyme. APRT catalyzs the conversion of the PP ribosylphosphate to adenine. In the complete absence of APRT activity, adenine is metabolized to 2,8 dehydroxyadenine (figure 15-1). Complete deficiency of APRT was described in 1974 [83]. The only clinical manifestations include urolithiasis, hematuria, urinary infection, obstruction or renal failure. Most patients develop kidney stones prior to four years to age [85]. In APRT deficiency, urinary stones are radiolucent and easily confused with uric acid. Urinary stones are composed of 2,8 dihydroxyadenine [84]. Serum uric acid concentrations are normal. Unlike uric acid, 2,8 dihydroxyadenine is more soluble in an acid urine [85].

APRT deficiency is inherited as an autosomal recessive trait [84, 109, 110]. Heterozygotes have erythrocyte APRT activity of 25–28% of normal but do not suffer from urolithiasis, and urinary excretion of 2,8 dihydroxyadenine is normal [110]. The incidence of heterozygosity is 0.4%–1.1% with the risk of APRT deficiency being approximately 1/100,000 [111]. The APRT gene locus is on the long arm of chromosome 16 [112]. A linkage between the APRT locus and the HPA gene that determines the alpha subunit of haptoglobin has been established [113]. The distance between these two loci on chromosome 16 has been estimated to be 27.5 centimorgans [110]. Therapy for patients with APRT deficiency includes a high fluid intake, a diet low in purines, and allopurinol to reduce the production of 2,8 dihydroxyadenine (figure 15-1).

HYPOURICEMIA AND UROLITHIASIS

Xanthinuria

Hypouricemaia is characteristic of xanthinuria, a rare, autosomal recessive disorder that has a high oxypurine excretion and xanthine oxidase deficiency. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine, as well as of xanthine to uric acid (figure 15–1). In humans, substantial xanthine oxidase activity is found in the liver and small intestine. Small amounts of xanthine oxidase activity are found in kidney, spleen, skeletal and cardiac muscle. Increased urinary xanthine is the result of both inhibition of degrada-

tion of xanthine to uric acid and the degradation of guanine nucleotides, which leads to enhanced hypoxanthine salvage [86]. Serum uric acid concentrations are quite low and range from 0.5 to 1.5 mg/dl. The urinary excretion of xanthine may exceed 500 mg/day.

Clinical manifestations of xanthinuria include urolithiasis, myopathy, and polyarthritis [88]. Approximately 30%–50% of patients with xanthinuria have urolithiasis [110]. Xanthine oxidase activity ranges from 0%–25% of normal. Diagnosis is based upon increased urinary oxypurine and xanthine excretion. Xanthine may represent 78%–80% of total urinary oxypurine in patients with xanthinuria [114]. Xanthine calculi are generally radiolucent; however, some xanthine stones contain sufficient calcium to become slightly opaque.

The gene encoding xanthine oxidase has not been identified. Pedigrees of families with xanthinuira are consistent with an autosomal recessive pattern of transmission [87–90]. Heterozygotes have demonstrated modest increases in urinary xanthine excretion with normal uric acid excretion [90]. Therapy for patients with xanthinuria includes encouraging a large fluid intake, low purine diet, and urinary alkalinization. Unfortunately, modifying urinary pH has a much more limited effect upon xanthine solubility than it does upon uric acid solubility (table 15-4).

Renal hypouricemia

Increased urinary uric acid excretion and urate stones may be associated with renal tubular dysfunction. In such patients, serum concentrations of uric acid may be quite low. Renal tubular dysfunction may be manifest by impaired tubular reabsorption of uric acid. Impaired renal tubular reabsorption of uric acid may be generalized to be presecretory or postsecretory. The most frequently documented defects in renal tubular reabsorption of uric acid are generalized or presecretory defects [91–105]. Uric acid urinary stones occur frequently in such patients. Fractional excretion of uric acid may be as high as 50%. In one family, four male siblings had renal hypouricemia [102]. An autosomal recessive gene lesion was postulated, although an X-linked gene defect could not be excluded. In this family the index case with hypouricemia was the product of a union of first cousins. Serum uric acid concentrations ranged from 0.6 to 1.1 mg/dl. While some affected individuals were asymptomatic, others had muscle wasting, weakness, dysarthria, and ataxia. Postsecretory defects in tubular reabsorption of uric acid have been suggested in some patients on the basis of normal inhibition of uric acid excretion by pyrazinamide, an inhibitor of uric acid secretion, and a reduced uricosuric response to benezomarone, an inhibitor of postsecretory urate reabsorption [91, 100, 101].

Hypouricemia and urate stones may also occur with increased secretion of uric acid [95, 103, 106]. Evidence for increased secretion of uric acid is based upon a renal uric acid clearance greater than creatinine clearance and correction or partial correction of the renal uric acid wasting with pyrazinamide, a pharmacological inhibitor of uric acid secretion. In patients reported to have

hypersecretion of uric acid, no additional renal abnormalities have been reported. Hypersecretion of uric acid has been reported in members of one family. Two sons and their mother were found to have extremely high renal urate clearance and low serum uric acid concentration [106]. In these individuals, pyrazinamide produced a marked reduction in uric acid clearance [106].

CYSTINURIA

Cystinuria is a recessively inherited disorder of amino acid transport characterized by excessive urinary excretion of cystine and the dibasic amino acids arginine, lysine, and ornithine, and by formation of renal stones. Clinical interest in the disease dates back to the early nineteenth century, when Wollaston reported "cystic oxide," an unusual component of urinary stones [115], later identified as cystine. Affected individuals were initially identified by the finding of flat, hexagonal cystine crystals during examination of the urinary sediment. The cyanide–nitroprusside test allowed chemical urinary cystine detection down to levels of approximately 150 mg/g creatinine, and this assay is still used a screening test today. More sensitive, quantitative methods of urinary amino acid analysis are now available. Ion exchange chromatorgraphy, paper chromatography, high-voltage electrophoresis, and even automated amino acid analyzers can be employed to obtain exact levels of urinary amino acids when the nitroprusside reaction is positive or equivocal [116, 117].

Cystinuria is an inborn error involving the specific membrane transport system for cystine, lysine, arginine, and ornithine. The incomplete tubular reabsorption of these amino acids is the direct result of transporter malfunction. In many patients, cystine clearance may exceed inulin clearance, suggesting the net secretion of cystine, while lysine clearance is usually 50%–70% of inulin clearance, and ornithine and arginine clearances are less affected [118]. At least three brush border transport systems are available to cystine and the dibasic amino acids: 1) a high-capacity (Km), low-affinity system for cystine alone, 2) a low-capacity, high-affinity system for cystine, lysine, arginine, and ornithine, and 3) a common system for lysine, arginine, and ornithine. The low Km, high Vmax common transport system, also present on intestinal mucosa, is postulated to exhibit a lower than normal Vmax, thereby limiting transport capacity [119, 120].

Although no intestinal disease occurs in cystinuria, defective intestinal mucosal uptake of cystine and the basic amino acids is well documented [121,122]. Cystinuric individuals fail to demonstrate a normal rise in plasma cystine following oral cystine load [123]. Using jejunal biopsy specimens obtained from cystinuric individuals, three patterns of defective intestinal transport have been described: type I has a complete deficiency of cystine and dibasic amino acid transport; type II lacks dibasic amino acid transport yet demonstrates a small amount of cystine accumulation; type III has variable uptake of the basic amino acids and cystine. Intestinal transport studies have been more useful than renal tubular transport studies in the assignment of phenotype and presumed genotype in cystinuria [14].

Table 15-5. Subtypes of cystinuria [117,126]

	Type I	Type II	Type III
Intestine			
In vitro transport	No transport of cystine, lysine, or arginine	No transport of lysine, minimal cystine uptake	Lysine transport present but reduced; cystine transport reduced to normal
Oral cystine load	No plasma cystine elevation	No plasma cystine elevation	Slow increase in plasma cystine to normal levels
Kidney			
Urinary amino acid excretion:			
Homozygotes			
Cystine	112–1212 mg/g creat	112–1212 mg/g creat	112–1212 mg/g creat
Lysine	296–4870 mg/g creat	296–4870 mg/g creat	296–4870 mg/g creat
Arginine	50–1842 mg/g creat	50–1842 mg/g creat	50–1842 mg/g creat
Ornithine	43–1579 mg/g creat	43–1579 mg/g creat	43–1579 mg/g creat
Heterozygotes			
Cystine	Normal	15–337 mg/g creat	13–19 mg/g creat
Lysine	Normal	73–745 mg/g creat	37–73 mg/g creat
Arginine	Normal	2–31 mg/g creat	3–4 mg/g creat

Cystinuria is inherited in an autosomal recessive pattern; however, intestinal transport studies and urinary excretion patterns of family members indicate three genetically distinct subtypes of cystinuria [124] (table 15-5). Family members of some cystinuric patients were found to be incompletely recessive since urinary excretion of cystine, lysine, and arginine were elevated. This apparent heterogeneity of phenotype expression is now attributed to the presence of three mutant alleles responsible for a variety of genotypes. Rosenberg has concluded that these three mutations are mostly likely at the same allele, since compound heterozygotes are phenotypically similar to homozygotes [125]. Three phenotypic groups can be identified, composed of combinations of three mutant alleles: 1) a cystinuric group composed of homozygotes and compound heterozygotes (I/I, I/II, I/III, II/II, II/III, III/III), 2) detectable heterozygotes (+/II, +/III), and 3) nondetectable heterozygotes (I/+).

Unfortunately, amino acid excretion patterns of stone patients do not reliably predict genotype. Differentation of homozygotes from heterozygotes is usually based on the urinary levels of cystine, lysine, and arginine in addition to the presence of cystine crystals or stones. Mean values for cystine in homozygotes is approximately 500 mg/g creatinine and is less than 400 mg/g creatinine in heterozygotes with a mean 337 mg/g creatinine in type II heterozygotes and 15 mg/g creatininine in type III. Urinary lysine concentration

ranges from 296–4870 mg/day (mean 1165 mg/g creatinine) in homozygotes, 73–745 mg/g creatinine (mean 338 mg/g creatinine) in type II heterozygotes, and 37–73 mg/g creatinine (mean 102 mg/g creatinine) in type III heterozygotes [126].

The assignment of genotype is based on urinary amino acid excretion patterns of obligate carriers as well as intestinal transport studies if needed. Extensive family studies [126–128] have allowed certain generalizations to be made based on urinary levels of cystine, arginine, and lysine in obligate carriers: type II heterozygotes usually display arginine levels of 10–50 mg/g creatinine, whereas type III heterozygotes have normal levels (0–3 mg/g creatinine). A five-fold ratio of lysine to cystine is helpful in distinguishing a type II heterozygote from a type III. Caution must be exercised when analyzing urine amino acid levels in infants less than six months of age. Scriver found that type II and III heterozygotes exhibited urinary cystine and lysine levels indicative of the homozygous state. These levels decreased to resemble the parental heterozygous phenotype by the age of six months [128].

The distribution of subtypes according to geographic location is variable. The completely recessive or type I form is twice as common in England and five times as common in Sweden [133] as type II and II combined. Type I accounts for approximately one half of cases reported in the United States. Of 15 families reported by Kelly [126], type I was present in 11: homozygous I/I in 6, and as a compound heterozygotes in 5. Homozygous III was present in one family, with a compound heterozygous state II/III found in two, and or uncertain III/? in one.

Cystinuria is reported as an etiology for urolithiasis in 1%–3% of stone-forming individuals. Cystine stones are yellow-brown, radioopaque calculi, and commonly contain other compounds such as calcium oxalate and magnesium ammonium phosphate [117]. The average age of stone presentation is the third decade or around 25 years of age [129]. A major factor promoting stone formation is the limited solubility of cystine as crystallization occurs at 300 μg/ml. Cystinurics commonly excrete 0.5–1.0 gram of cystine per 24 hours or 500–1000 ng/ml cystine. Cystine solubility increases two-fold when urinary pH exceeds 7.5 [117]. Ettinger and Kolb found enhanced cystine solubility in a concentrated urine (sp. gr. > 1.010) compared to more dilute urine and speculated that other factors besides cystine concentration affected crystallization [131]. Not all homozygous cystinurics develop urinary calculi. Dahlberg reported that 55% of their series of 89 cystinuric patients formed documented cystine stones, while an additional 13% formed primarily calcium-containing stones [130]. Clearly, elevated urine cystine levels alone cannot account for stone formation. Other metabolic factors possibly contributing to stone formation in Dahlberg's series were hyperuricemia, hyperuricosuria, and hypercalciuria. Urinary citrate levels were not measured.

Recent studies have revealed an increased incidence of heterozygous cystinuria among stone patients when compared to control populations, suggesting

Table 15-6. Frequency of +/II and +/III cystinuria among stone-forming individuals

Number of cases	Frequency	Reference
17/126	.135	Resnick [132]
6/150	.040	Giugliani [134]
5/51	.078	Thomas [135]

that the heterozygous type II or type III individual is at risk for urolithiasis (table 15-6). Of the 126 patients with renal stones studied by Resnick et al. [132], 17 were heterozygous for cystinuria (2 type II, 15 type III). Further metabolic evaluation disclosed hypercalciuria and/or hyperuricosuria in 50%. Gugliano [133] screened 200 stone patients from Brazil and detected one type I homozygote and six heterozygotes. The heterozygous gene frequency in stone populations is estimated to be between 0.04 and 0.13 [132–135], whereas the gene frequency in control populations is 0.001 to 0.009 [133]. Factors responsible for stone formation in heterozygotes are not understood. Contribution by secondary metabolic factors or the absence of protective substances may promote stone formation in these individuals. Certainly, the type II or II heterozygous state can no longer be viewed as a clinically innocuous one.

Therapy for cystinuria includes aggressive urinary alkalinization and a large fluid intake to produce a hypotonic diuresis. When recurrent stones persist despite these measures, D-penicillamine is helpful in reducing the risk for urolithiasis. Penicillamine combines with cystine to form a more soluble cysteine disulfide complex. Unfortunately, D-penicillamine has a large number of adverse effects, including gastrointestinal discomfort, loss of taste perception, bone marrow depression, proteinuria, membranous glomerulopathy, optic neuritis, myasthenia gravis, and trace metal deficiencies. Recently, a new, less toxic agent, mercaptopropionylglycine (MPG), has become available to reduce urinary stone formation in patients with cystinuria. MPG reduces urinary cystine crystallization by a mechanism similar to D-penicillamine. A disulfide exchange reaction produces a more soluble sulfur compound. Adverse effects of MPG seem to be mild: skin rash, nausea, and fever [116].

CONCLUSIONS

In the vast majority of patients with urolithiasis, the gene defect(s) or mode of inheritance has not been elucidated. Table 15-7 summarizes the genetic basis of the most common etiologies of urolithiasis. As our understanding of the precise pathogenetic mechanisms of urolithiasis increases, determination of the genetic basis of urolithiasis will be possible, and genetic probes to identify individuals at risk may then be developed. Such identification will then allow preventative measures to avoid clinical urinary stone formation or, possibly, to permit correction of the gene defect.

Table 15-7. Summary of conditions causing urolithiasis

Condition	Genetic transmission
Idiopathic calcium oxalate urolithiasis	Polygenic, autosomal dominant
Idiopathic hypercalciuria	
Absorptive	?Autosomal dominant
Renal	?Autosomal dominant
Renal tubular acidosis type I	Sporadic; autosomal dominant (rare)
Hyperparathyroidism	
Adenoma	Autosomal dominant
MEN type I and II	Autosomal dominant
Hyperuricemia	
Primary gout	Polygenic
Hypoxanthine–guanine	X-linked
Phosphoribosyl transferase deficiency	
Hypouricemia	
Xanthine oxidase deficiency	Autosomal recessive
Renal urate wasting	Sporadic; autosomal recessive (rare)
Adenine phosphoribosyl transferase deficiency	Autosomal recessive
Cystinuria	
Type I	Autosomal recessive
Type II	Autosomal recessive
Type III	?Autosomal recessive
Hyperoxaluria	
Type I	Autosomal recessive
Type II	Autosomal recessive

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16. GENETICS OF PRIMARY HYPEROXALURIA

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Primary hyperoxaluria (PH) is a rare inborn error of metabolism characterized by increased production of oxalate and glycolate [1]. Urinary excretion of oxalate always exceeds 100 mg (1.1 mmol), and usually 200 mg (2.2 mmol) per day, as compared to less than 45 mg (0.5 mmol) in normals [1]. Elevated urinary excretion of oxalate not only leads to formation of calcium oxalate stones and to repeated attacks of renal colics, but also results in crystal deposition in the renal interstitium, which induces fibrosis and nephrocalcinosis. Renal damage, in turn, leads to oxalate retention and involvement of other organs, primarily of the bones, the arteries, the cardiac conduction system, the retina, and the neuromuscular system. Two types of PH have been described, of which type I (glycolic aciduria) is much more common than type II (L-glyceric aciduria) [2]. The discussion will therefore focus on PH type I. Apart from these two types, there appears to exist yet another variety of PH, in which the urinary excretion of both glycolate and glycerate are normal [3–5].

PRIMARY HYPEROXALURIA TYPE I (GLYCOLIC ACIDURIA, MCKUSICK 25990)

The clinical spectrum of this condition is very wide. Most patients have repeated episodes of renal calculi starting in early life. Diagnosis is often missed at this stage, being frequently made only after the onset of renal insufficiency. End-stage renal failure (ESRF) may be observed at any age, from early infancy

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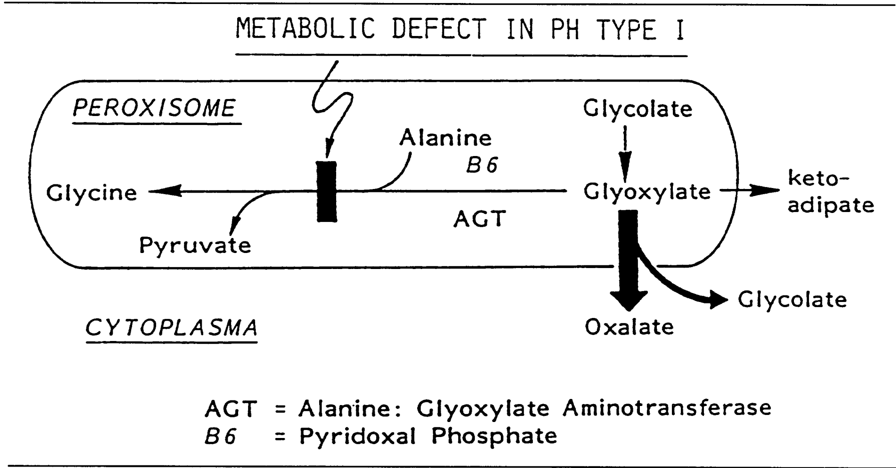

Figure 16-1. The metabolic defect in primary hyperoxaluria type I.

until late adulthood. In early reports, the majority of the patients (up to 80%) were younger than 20 years of age [1,6] whereas in more recent surveys, one from Sweden [7] and another from Switzerland (Colombi and Leumann, personal communication; [8]), the majority (61% of 41 patients) were older than 20 years of age. In a small subgroup of patients, the disease becomes manifest clinically during the first few weeks of life and runs a particularly severe course, leading to early renal failure, in the absence of urolithiasis [9]. Only 30 cases of this so-called infantile oxalosis have so far been described in detail [9].

PH was observed in different ethnic groups. Its overall frequency is not known. In the German part of Switzerland (approximate population four million), 24 patients with PH (from 16 different families) were detected between 1972 and 1988.

PH type I is due to deficiency of the peroxisomal enzyme alanine: glyoxylate aminotransferase (AGT) [10] (figure 16-1), and not to the cytosolic 2-oxo-glutarate: glyoxylate carboligase, as had been suggested in the past [1]. As a result, the glyoxylate produced in the liver peroxisomes cannot be transaminated to glycine [10]; instead, the glyoxylate passes into the cytoplasm, where it is transformed into oxalate and glycolate (figure 16-1). PH type I should therefore be added to the list of peroxisomal disorders [10]. The residual AGT hepatic activity appears to correlate with the oxalate production rate and thus, in part, with the clinical expression of the disease [10]. The level of residual AGT activity might therefore serve as a prognostic indicator. However, severe renal insufficiency is often precipitated by extrarenal factors, primarily by episodes of dehydration or surgery [11].

The genetic and biochemical heterogeneity of the disease explains the vari-

able effect of pyridoxine administration. Only approximately one third of patients have been shown to respond to this treatment, as demonstrated by a reduction of urinary oxalate excretion [1, 3, 5, 12, 13]. Pharmacological doses of pyridoxine (300–600 mg daily) are usually required, but a few patients have responded to physiological doses (2–20 mg daily) [5]. The partial pyridoxine dependency observed in PH type I is explained by a defect in transamination [10], for which pyridoxal phosphate is the cofactor (figure 16-1).

Inheritance

In the vast majority of cases, PH type I is inherited as an autosomal recessive trait. This is evidenced by the following observations:

1. There are numerous reports of families with more than one affected child, yet most cases occur sporadically.
2. Parental consanguinity has repeatedly been noticed [6, 14, 15].
3. There is no significant difference in sex incidence [1, 6, 15], although in some series a male preponderance has been observed: 13 male versus 4 female patients in a Scandinavian survey [7] and 17 male versus 7 female patients in a Swiss survey (Colombi and Leumann, personal communication).

Parents and nonaffected siblings of patients with PH type I usually have normal urinary excretion of oxalate (and glycolate) [1]. Detection of heterozygotes is therefore not possible by examination of biological fluids. However, the determination of AGT enzyme activity from liver biopsy appears to allow diagnosis in carriers, since intermediate levels were found in one case [16].

Autosomal dominant inheritance of PH is suggested by a few families in which a parent and the offspring were affected [4, 17–20]. However, some of these reports are old, and the documentation is sometimes insufficient. In all these families, the oxalate excretion in the parent was only moderately increased (80–110 mg per 24 hours). Such excretion rates are below those found in typical cases of with PH type I. However, some heterozygotes might have only a slightly raised oxalate excretion; further family studies are necessary to resolve this issue. Autosomal dominant inheritance appears to exist in a Swiss family observed by Reutter (personal communication). PH was diagnosed in the propositus only after he developed terminal renal failure at the age of 50 years. His brother and four of his children had oxalate excretion rates between 105 and 340 mg per day, yet only slightly high glycolate excretion (90–140 mg per day). Enzyme determinations have not been done so far, and therefore it remains unknown whether this family, and that reported by Holmgren et al. [4], have type I PH or a variant. Finally, pseudodominance, i.e., marriage of an affected homozygote with a heterozygote, results in 50% homozygotes and thus, in rare instances, may result in affected offspring.

Several reports mention a high incidence of urolithiasis in families of patients with PH type I [4, 6, 7, 14], but a relationship to increased oxalate excretion has not been established. Thus, at present there is no convincing evidence for the existence of a subgroup of patients with clinical features and course similar to PH type I but with autosomal dominant inheritance.

Genetic counseling

The purpose of genetic counseling in PH type I is two-fold: first, to diagnose or exclude the disease in siblings, and second, to inform the parents and patients about the prognosis and the risk of disease in the progeny. The recurrence risk in siblings of an affected child is 25%, irrespective of sex; unfortunately, diagnosis in patients with the classic form of PH is often made only after the age of 10 years or, sometimes, even in adulthood. In affected siblings the prognosis tends to be better than in the propositus, thanks to earlier treatment and to the awareness of the risks of dehydration. Genetic counseling is of particular importance to families with severely affected patients, primarily to those with infantile oxalosis [9]. The 25% risk of having another child with serious illness may justify an attempt to establish the diagnosis prenatally. The risk of an affected individual having an affected offspring is very low, because the risk that the apparently non-affected spouse is heterozygous is negligible, unless he/she is either consangineous with the patient or from another PH family.

Clinical course

The course of the disease in siblings of the same family may be quite different, although the genetic defect must be identical. This diversity is due mainly to nonrenal manifestations of the disease and has been seen even in families with infantile oxalosis. McCredie (personal communication) observed a family in which the first child presented in terminal renal failure at the age of five months, whereas his younger brother, who had an episode of ureteric obstruction at birth and was then treated symptomatically, developed rapidly progressive renal failure at the age of 11 years. In a Tunisian family, one affected girl died of renal failure at one month of age, whereas her three affected sisters aged 4, 14, and 16 years were alive at the time of the report [14].

Prognosis and treatment

Prognosis depends greatly on the response to pyridoxine administration. If renal function is not seriously impaired at the time treatment is started and the therapy is effective, survival into adult life with no or rare colicky pain may be expected. Once renal failure has ensued, the prognosis is grim. Neither hemodialyis nor peritoneal dialysis is able to remove adequate amounts of oxalate [21], and generalized deposition of calcium oxalate inevitably occurs. The only intervention that can alter this course is renal transplantation. Unfortunately, recurrence of oxalosis in the homograft occurs almost uni-

formly [8, 22], making most physicians reluctant to recommend renal transplantation. This is particularly true for the infantile form, although one group has reported satisfactory results [23]. The high risk of recurrence also explains the reluctance to use kidneys from live donors (mainly from parents), despite the fact that the outcome of these patients is better than that of those who receive cadaver kidneys [23]. The better results observed in patients with live related donors are due to the intensive dialysis to which they are subjected for a few days prior to transplantation [23] and to the substantially lower risk of acute tubular necrosis that is associated with rapid deposition of oxalate. From a metabolic point of view, there is no objection to kidney donation from obligatory heterozygotes (parents). Recently, combined liver and kidney transplantation has been performed in a patient with PH type I, leading to correction of the metabolic defect [24].

Prenatal diagnosis

Measurement of oxalate and glycolate in amniotic fluid obtained at 16 weeks of gestation has failed to diagnose the disease [25]. The concentrations of both acids were within the range found in the amniotic fluid of 13 apparently normal women at 15 to 18 weeks of pregnancy [25]. This finding was not unexpected, since both oxalate and glycolate are thought to pass through the placenta and thus to be cleared from the fetus. Diagnosis of PH by assaying the activity of alanine: glyoxylate aminotransferase (AGT) in amniotic fluid or chorionic villi is not possible, because this enzyme is expressed almost exclusively in the liver [26]. Prenatal diagnosis of PH type I by demonstration of a abnormal AGT activity in hepatic tissue obtained by fetal biopsy has recently been reported [27]. Based on the result of this test, the fetus was aborted a few days later and the diagnosis was confirmed. It is obvious that this procedure requires special skills and equipment and should therefore be restricted to specialized centers.

Because the abnormal gene product has been identified [10], recombinant DNA technology and DNA analysis might in the future allow prenatal diagnosis of PH I by chorionic villus sampling. For this to occur, the gene will have to be localized, and intra- and/or closely linked extragenic restriction fragment length polymorphisms will have to be established. This method should permit a definitive diagnosis as early as the 11th or 12th week of gestation, with a lower risk of abortion than that likely to occur after liver biopsy.

PRIMARY HYPEROXALURIA TYPE II (L-GLYCERIC ACIDURIA, MCKUSICK 26000)

This disease was originally reported in 1968 in four patients, three of whom were siblings [2]. All patients had intermittent microscopic hematuria due to calcium oxalate urolithiasis. A progress report on these cases was published 15 years later [28]. The oldest patient had undergone right nephrectomy and the

second had started hemodialysis at the age of 23 years, whereas the last one, aged 18 years, maintained normal renal function. Only one other report on PH type II has appeared since [29]. It contains the description of brothers in two unrelated families affected by recurrent urolithiasis. Two of these patients were over 20 years old, but only one, who had suffered a right nephrectomy, had reduced renal function [29]. Type II of PH is thus far less common than type I, although diagnosis may occasionally have been missed [29]. It is not clear how the metabolic defect [2], a deficiency of the enzyme D-glycerate dehydrogenase, relates to the increased urinary excretion of oxalate [30]. The prognosis appears to be fairly good, particularly when the diagnosis is made early. The finding of normal urinary and plasma organic acids in the parents and three siblings from one family [29] suggest an autosomal recessive mode of inheritance.

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IV. SYSTEMIC DISORDERS

17. HERITABLE MALFORMATIONS OF THE KIDNEY AND URINARY TRACT

ENID F. GILBERT-BARNESS, JOHN M. OPITZ, LEWIS A. BARNESS

A malformation is defined as a morphological defect of an organ, part of an organ, or a larger region of the body resulting from an intrinsically abnormal developmental process [1].

Inheritance of malformations can be classified in several ways. For the purposes of this chapter, we follow the classical genetic modes of inheritance: autosomal dominant, autosomal recessive, and X-linked recessive. Sporadic malformations for which information regarding inheritance is lacking are listed on a table (table 17-4). The renal lesions found in chromosomal defects are listed separately because their inheritance pattern is variable: most trisomies are sporadic, due to nondisjunction; translocation abnormalities may be transmitted through a balanced translocation-parent carrier. Functional abnormalities without obvious structural malformations are described in other sections of this volume.

Specific renal malformations are relatively rare in dysmorphic syndromes. Examples include the common association of horseshoe kidney in gonadal dysgenesis (Turner syndrome) and chromosome 18 trisomy, and hypoplasia of the kidney in obstruction sequences. However, variable renal anomalies and renal malfunction are integral parts of many syndromes, including those with clearly defined modes of inheritance. Some of these conditions, such as obstructive uropathy [2, 3] can be identified in utero by ultrasonography, while those associated with enzymatic or chromosomal defects can be diagnosed by examination of the amniotic fluid or chorionic villi.

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RENAL DYSPLASIA

Dysplastic kidneys are the result of an abnormal differentiation of the metanephros. Typical dysplastic features are the presence of metaplastic cartilage, primitive ducts, and lobar disorganization [4, 5]. Metaplastic cartilage appears within the cortex as bars and nests of hyaline cartilage. Primitive metanephric ducts, which may be cystic, are lined with undifferentiated epithelium and surrounded by fibromuscular collars. Abnormal corticomedullary relationships and rudimentary medullary development constitute lobar disorganization. These abnormalities bear a strong relationship to urinary tract malformations [6, 7], including ureteral atresia and urethral valves, suggesting that urinary obstruction or urinary reflux during metanephric development leads to renal dysplasia [4].

Dysplastic kidneys are often cystic; the most common variety perhaps is the multicystic dysplastic kidney [8]. The enlarged misshapen, irregularly cystic kidney is closely related to aplastic dysplasia—the small, barely recognizable, rudimentary, solid nubbin. The difference is in the degree of cyst formation. All degrees intermediate between the two prototypes exist. Some multicystic kidneys contain masses of undifferentiated cells, which have been referred to as nodular blastema [8, 9]. Nodular blastema may be related to the rare development of Wilms' tumors [9] or renal cell carcinoma [10, 11].

The multicystic dysplastic kidney is usually detected in the newborn as a flank mass, and sonography shows large, spherical cysts with nondelineation of the renal sinus [12, 13]; frequently there are renal and urinary tract abnormalities contralaterally [14, 15]. Malformations of other systems, especially congenital heart disease and esophageal or intestinal atresia, are common.

Diffusely cystic dysplastic kidneys with patent urinary tracts occur principally in malformation syndromes. They should not be confused with multicystic kidneys, since the clinical and genetic implications are different. The cysts in diffuse cystic dysplasia typically arise within primitive collecting ducts. There may be a striking paucity of nephrons. Clusters of glomeruli and convoluted tubules are present among the cysts. Cartilage is seldom present. Diffuse cystic dysplasia occurs with regularity in Meckel syndrome [16, 17], and less often in a group of disorders that includes several forms of short-limbed chondrodysplasia, Zellweger syndrome, glutaric aciduria type 2, and renal–hepatic–pancreatic dysplasia [18]. In all these syndromes, the liver contains a biliary abnormality very similar to that of autosomal recessive polycystic kidney disease and congenital hepatic fibrosis. Specific diagnosis depends on recognition of the syndrome, since the renal abnormality is similar in all of them.

The risk of inheritance of renal dysplasia is very small [20]. Multicystic and aplastic kidneys are inherited, usually in a dominant pattern, in the hereditary renal adysplasia syndrome, which includes unilateral dysplasia and unilateral agenesis [20–22]. There is also a small risk of obstructive dysplasia due to the familial occurrence of posterior urethral valves [23]. The risk of recurrence of

diffuse cystic dysplasia is generally greater, but varies from one associated syndrome to another.

Lobar or lobular dysplasia is a focal segmental dysplasia characterized by the presence of isolated bars of metaplastic cartilage or of rudimentary lobes or lobules in the middle of an otherwise normally developed kidney; it is found in some autosomal malformation syndromes [24].

In a survey of congenital malformations associated with renal dysplasia and renal hypoplasia, Rubenstein et al. [25] found that 28 of 31 (90%) patients with renal dysplasia but only 7 of 58 (12%) patients with renal hypoplasia had other urinary tract malformations. Unilateral dysplasia is associated with ipsilateral ureteral maldevelopment, while bilateral dysplasia is associated with lower tract obstruction. Furthermore, segmental dysplastic abnormalities are related to the dependent ureter, as in duplex kidneys with ureteral duplication. However, patent urinary tracts, without evidence of urinary tract obstruction or vesicoureteric reflux, are observed in approximately 10% of sporadic cases of dysplasia. Hereditary and syndrome-associated dysplasias are usually not accounted for by urinary tract malformations.

There is a certain degree of correlation between the type and severity of obstruction and the degree of dysplasia [4]. For instance, cortical dysplasia is found in young infants with severe obstruction and clinically apparent renal disease, but not in relatively asymptomatic older children and adults with milder urinary obstruction.

The occurrence of dysplasia in only four of the patients with hereditary syndromes such as Zellweger and Jeune indicates the multifactorial origin of these conditions. It has been suggested that only genetic defects expressed early during ontogeny may lead to abnormal renal development and account for dysplasia [26].

The risk of recurrence is much greater in syndromatic dysplasia. Many autosomally inherited syndromes include, with variable frequencies, aplastic and multicystic kidneys, as found for example in the VATER association and in the branchio-oto-renal syndrome. Diffuse cystic dysplasia occurs often in malformation syndromes [18].

The renal abnormalities, being similar in all these conditions, provide little clue to the differential diagnosis, which depends upon identification of other abnormalities.

AUTOSOMAL DOMINANT MUTATIONS

Renal lesions and associated abnormalities in autosomal dominant mutations are shown in table 17-1.

Bilateral renal agenesis (BRA), Unilateral renal agenesis (URA), and renal adysplasia (RAD)

The most common Mendelian mutation in which BRA/URA is seen is autosomal dominant hereditary renal adysplasia (HRA), first described

Table 17-1. Autosomal dominant mutations

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Bilateral renal agenesis (BRA), unilateral renal agenesis (URA), hereditary renal adysplasia (HAD)	Unilateral agenesis with contralateral hypoplasia or dysplasia, bilateral renal agenesis	None
Tuberous sclerosis	Cystic kidneys, renal angiomyolipomas	Epilepsy, mental retardation, hamartomas, skin lesions, angiofibromas, intraventricular calcifications
von Hippel–Lindau (VHL) disease	Cystic kidneys, cytoadenomas of epididymus, renal carcinoma, pancreatic cysts	Cerebellar and retinal angiomyolipomas
Peutz–Jeghers syndrome	Adult polycystic kidney disease	Intestinal polyposis, mucosal pigmentation
Familial hydronephrosis	Unilateral or bilateral hydronephrosis with or without ureteroplevic obstruction, contralateral renal agenesis	None
Alagille syndrome (Arteriohepatic dysplasia)	Marked arterionephrosclerosis with diffuse calcinosis, single kidney, small kidneys, and renal artery	Distinctive phenotype, vertebral anomalies (butterfly vertebrae), peripheral pulmonary stenosis, mental and growth retardation, neonatal cholestasis
Nail-patella (hereditary onycho-osteodysplasia) syndrome (NPS)	Thickening of glomerular basement membranes, mesangial hypercellularity, glomerular sclerosis with tubular atrophy and interstitial fibrosis, segmental sclerosis, cortical atrophy and chronic nephritis, focal deposits of IgM and/or complement. Collagen fibers within basement membrane of glomeruli, collagen fibers in mesangium	Onychodysplasia, fingers and toes, small or absent patellae, iliac spurs, iris heterochromia, malformation of radius
Branchio-oto-renal syndrome (BOR)	Sharply tapered superior poles and blunting of calyces, hypoplasia	Preauricular pits, branchial fistulas, hearing loss
Opitz–Frias syndrome (G or dysphagia hypospadias syndrome)	Duplication of renal pelvis and ureters, bilateral ureteral reflux	Deglutition difficulty, stridorous respiration, weak, hoarse cry, hypertelorism, hypospadias, abnormalities of laryngotracheobronchial tree
Townes–Brocks syndrome (thumb, auricular, anal, and renal anomalies)	Renal hypoplasia, ureterovesical reflux, and posterior urethral valves	Thumb, auricular, and anal anomalies, congenital heart defects, anomalies in external organs
Myotonic dystrophy and polycystic kidney disease	Adult type polycystic kidneys	Cleft lip and palate, congenital heart defect, deafness, epilepsy, malformations, limb neurofibromatosis, eye defects

Table 17-1 (continued)

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Ectrodactyly–Ectodermal dysplasia–cleft (EEC) syndrome	Unilateral renal agenesis	Cleftlike palate, ectodermal dysplasia, deafness, small malformed ears
Ochoa syndrome	Hydronephrosis and hydroureter, intravesical stenosis of ureter, abnormal caliber of the urethra, urethral valves	Peculiar facies
Brachmann–de Lange syndrome*	Hypoplastic, cystic, and dysplastic kidneys	Shortness of stature, mental retardation, microbrachiocephaly, bushy eyebrows and synophys, hirsutism phocomelia, cardiac and G.I. defects
Familial amyloid nephropathy (Andrade syndrome with polyneuropathy; Van Allen syndrome with neuropathy and peptic ulcer)	Amyloid nephropathy vascular renal atrophy due to renal amyloidosis	Neuropathy involving lower limbs, peptic ulcer

*Familial occurrence, but autosomal dominant in some cases seems likely. Empiric recurrence risk low.

by Buchta et al. [20]. In HRA, one kidney is absent and the other is hypoplastic dysplastic (figure 17-1, A and B). BRA is uncommon among heterozygotes, even though it is the usual mode of ascertaining the presence of the hereditary defect in families with URA and adysplasia (figure 17-2); however, the trait is frequently nonpenetrant. Twins may be concordantly [27] or discordantly affected. First- and second-degree relatives should be examined ultrasonographically.

The prevalence of BRA, URA, or RAD was reported by Roodhooft et al. [28] to be one case in 6369 births. Among 41 affected infants, 21 had BRA, 10 had RAD, and 10 had bilateral dysgenesis. One hundred and eleven first-degree relatives (71 parents and 40 sibs) had renal ultrasonography. Of these, 12% (n = 13) had *silent* genitourinary anomalies, and 9% (n = 10) had kidney malformations, including URA (n = 5), double ureters (n = 2), hydronephrosis (n = 1), multicystic kidney (n = 1), and multiple renal cysts (n = 1). The frequency of renal malformations among parents and sibs was similar in all three groups of index patients. Among 682 control individuals who had abdominal ultrasonography, only two (0.3%) had URA, a rate 14–17 times lower than that among the parents and sibs of the patients. In one of the families, one infant had BRA, a sister and their mother had URA, and the mother's two sisters had pelvic kidneys and unilateral absence of uterus,

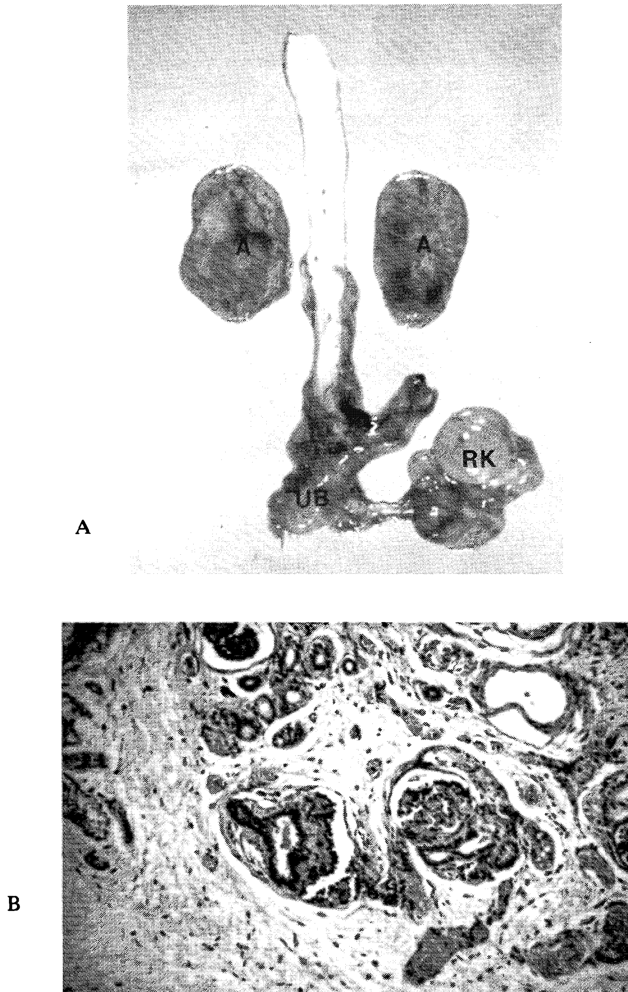


Figure 17-1. Renal adysplasia. **A.** The right kidney (RK) is ectopic in the pelvis and is dysplastic. There is agenesis of the left kidney. The adrenal glands (A) are large and disc-shaped. The urinary bladder (UB) is hypoplastic. **B.** Hypoplastic dysplastic kidney—microscopic appearance.

ovary, and fallopian tubes. In the sample population studied by Roodhooft et al. [28], 30% of parents with two affected infants had a silent renal malformation. Thus, a substantial proportion of patients with BRA have renal adysplasia. Because Bernstein et al. [29] and Kornguth et al. [30] have documented a developmental relationship between brain and kidneys, we recommend a careful analysis of CNS in all BRA/URA autopsy cases.

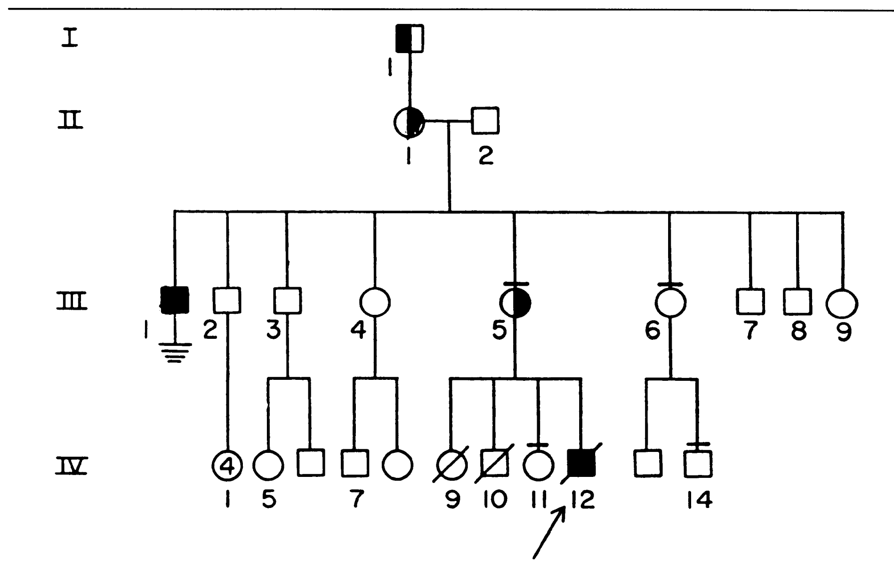


Figure 17-2. Hereditary renal adysplasia: Pedigree of family with unilateral renal agenesis (URA), bilateral renal agenesis (BRA), and renal adysplasia (RAD). I-1, II-1, and III-5 had URA. III-1, a stillborn male, had BRA; IV-12 died shortly after birth and had RAD.

Tuberous sclerosis

Tuberous sclerosis (TS) is a relatively common autosomal dominant disease characterized by epilepsy, mental retardation, and a variety of skin manifestations, including shagreen patches, ash-leaf-shaped spots, and angiofibromata. Intracerebral calcifications and hamartomas of the viscera, particularly the kidneys (figure 17-3), heart, lungs, and spleen, occur in over 50% of the cases. The renal manifestations may precede other clinical signs of the syndrome [31, 32].

The renal lesion of TS is characterized by cysts lined with hyperplastic epithelium forming papillary and polypoid masses that protrude into the cystic spaces. It has been suggested by Bernstein and Kissane [29] that cellular hyperplasia is the cause of renal tubular enlargement and cyst formation. Microdissection in one newborn infant [33] demonstrated that the thin-walled cysts were enlargements of Henle's loops. Subcapsular cysts surrounded by connective tissue arose from collecting tubules. We have observed one case in which the renal involvement was grossly similar to the adult polycystic disease. Cystic renal disease is less frequent in TS than solid angiomyolipomatous hamartomas (50% of cases), which are usually small and numerous. Symptoms are variable and include renal enlargement without urinary abnormalities [34], early onset of hypertension [35], and rarely renal insufficiency [32]. There appears to be an increased risk of renal carcinomas, perhaps because of the epithelial hyperplasia.

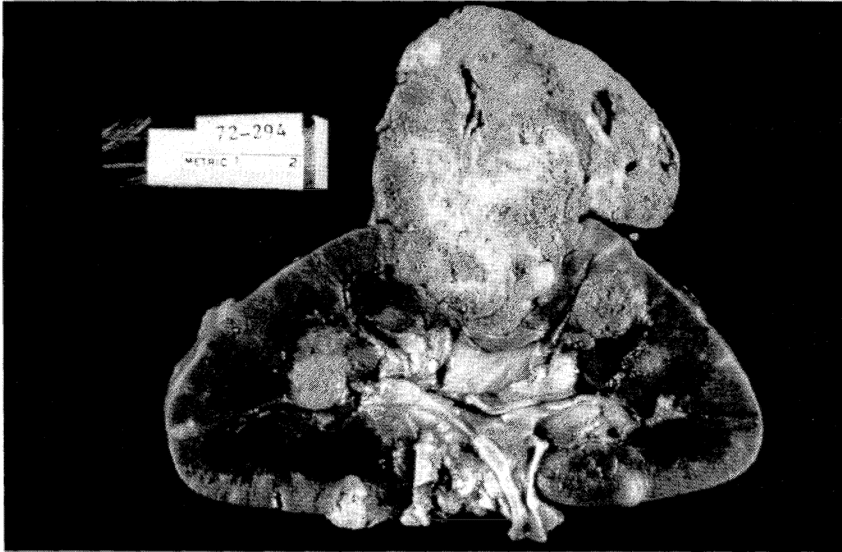


Figure 17-3. Multiple angiomyolipomata of kidney in tuberous sclerosis.

Von Hippel–Lindau (VHL) dysplasia

Von Hippel [36] observed angiomatosis retinae, and Lindau [37] described cerebellar angiomatosis associated with a variety of visceral lesions. The visceral components of this complex are most frequently cysts of the kidney and cysts or cystadenomas of the pancreas or epididymus. Renal cysts are present in approximately two thirds of the cases, and pancreatic cysts in about one half. Renal cysts can be diffuse, resembling autosomal dominant (adult) polycystic disease (figure 17-4), or can be highly localized. It has been hypothesized that the lesions represent an abnormality in the integration between blood vessels and parenchyma [38], but the cysts are notably associated with epithelial hyperplasia. The hyperplasia may be responsible both for the cysts, by causing tubular and ductal obstruction, and for the frequent development of renal-cell carcinomas. The tumor may appear in as many as 40% of the patients by the fourth and fifth decades of life. Many tumors are intracystic, taking the forms of mural nodules or polyps lined with neoplastic cells (figure 17-5). The condition is inherited as an autosomal dominant trait with an estimated penetrance of 80%–90%.

Peutz–Jeghers syndrome

Kieselstein et al. [39] described polycystic kidneys of the adult type in five adult brothers who also had the Peutz–Jeghers syndrome. Both disorders are known to be dominantly inherited, and their occurrence in this family may be a coincidence or a true syndromal association [40].

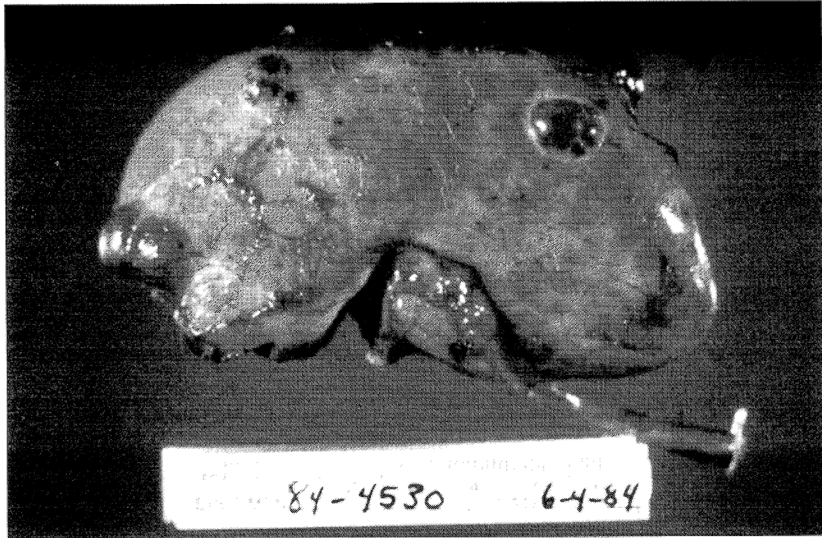


Figure 17-4. von Hippel–Lindau disease. Gross appearance of cystic kidneys. (from: Gilbert EF, Opitz JM: Chapter 64, Renal Involvement in Genetic–Hereditary Malformation Syndromes. In: Hamburger J, Crosnier J, Grunfeld JP (eds): Nephrology. New York: John Wiley & Sons, 1979, pp. 909–944).

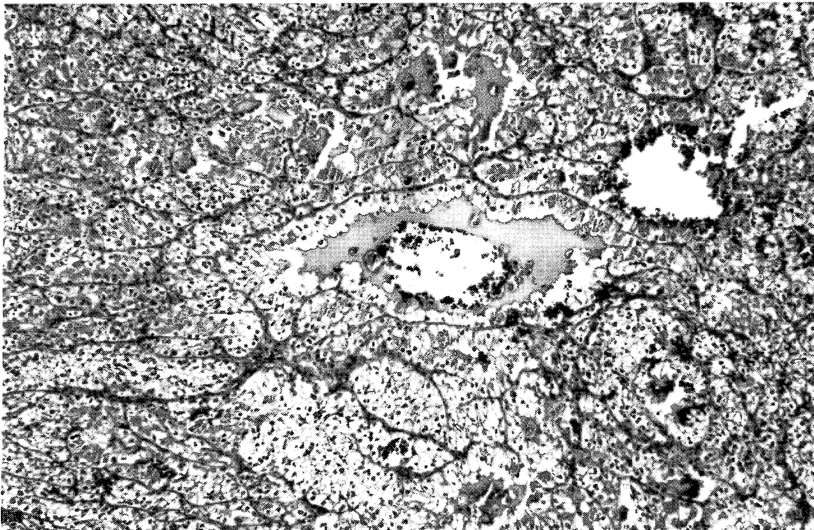


Figure 17-5. Clear-cell carcinoma arising from renal cyst in von Hippel–Lindau disease. H&E Mag X250.

Hydronephrosis, familial

The familial occurrence of unilateral or bilateral hydronephrosis has been described [41]. Transmission of the trait from a parent to one or more offspring implicates autosomal dominant inheritance. Both sexes are equally affected, although the condition appears to be more severe in males. Both unilateral and bilateral hydronephrosis in a single family can be viewed as differing expressivity of the gene, although genetic heterogeneity cannot be excluded [40]. The condition has been described in identical twins [42]. Ureteropelvic obstruction was found in some cases; in others no apparent cause for the hydronephrosis has been found. Faulty muscle development or absence of ganglion cells is a more likely cause of the hydronephrosis than an extrinsic defect such as fibrous bands or aberrant vessels [41]. In four families an association of either unilateral hydronephrosis with contralateral renal agenesis [43, 44] or a nonfunctioning kidney in a family member [41, 45] has been observed. This suggests that renal agenesis or aplasia may represent the most extreme form of this disorder, which in its less severe form is manifested by either functional or anatomic ureteropelvic obstruction with hydronephrosis [40] (see also Schintzel–Giedion syndrome).

Alagille syndrome

Arteriohepatic dysplasia (AHD) was first described by Watson and Miller [46], but the full constellation of this syndrome was delineated by Alagille et al. [47]. The characteristic features include distinctive facies with hypertelorism, prominent forehead, straight nose, and narrow pointed chin; vertebral anomalies, most often butterfly vertebrae; a heart murmur, usually due to peripheral pulmonic stenosis; frequently both mental and growth retardation; and neonatal cholestasis with a paucity of bile ducts on liver biopsy [48] and prominent Schwalbe's line. Parent-to-child transmission in some pedigrees is consistent with autosomal dominant inheritance [49–53].

Renal abnormalities have included marked arterionephrosclerosis with diffuse calcinosis, single kidney, small kidneys, and renal artery stenosis.

Nail-patella syndrome (NPS) (hereditary onycho-osteodysplasia)

Some 40% of patients with this autosomal dominant condition, which is linked to the ABO locus, have renal disease, and 10% die of renal failure [54].

Onychodysplasia of fingers and toes includes hypoplasia, longitudinal ridging, and hemiatrophy. The nails of the thumbs and great toes are the most severely involved. The patallae are small and sometimes absent. The radial capitellum may be malformed and may impair extension of the forearms, often accompanying dislocation of the radius. Most patients have iliac osseous spurs described as *horns*; these extend posteriorly from the iliac wings. Other bone disorders may occur, as well as a peculiar heterochromia of the iris [55, 56]. Hypertension, cylindruria, and hematuria have been reported. How-

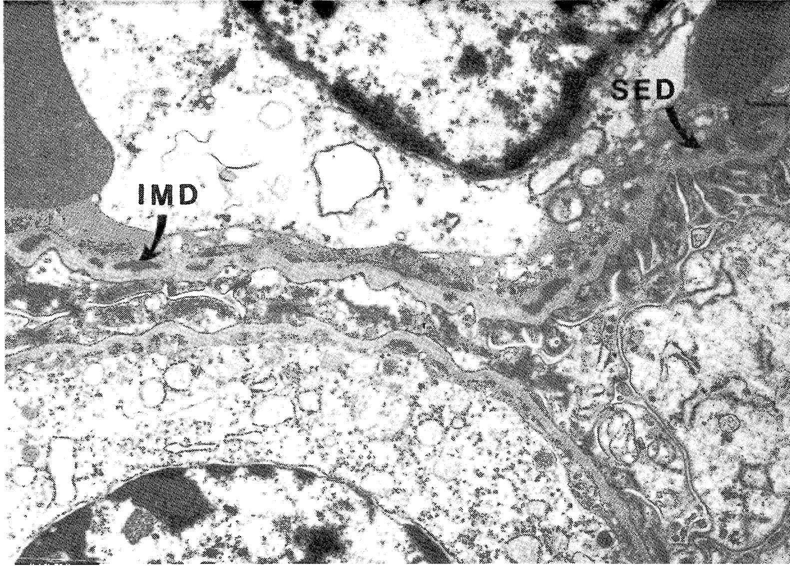


Figure 17-6. Nail-patella syndrome. Thickened capillary walls with numerous intramembranous deposits (IMD) and subendothelial electron-dense deposits (SED) that correspond to fibrinogen seen by immunofluorescene. Mag X16,500.

ever, proteinuria often resulting in nephrotic syndrome seems to be the most common renal abnormality.

The histological findings have been reviewed by Del Pozo and Lapp [57]. The renal lesions vary and depend on the age of the patient. Light microscopy and electron microscopy have sometimes shown thickening of the glomerular basement membrane [58–61]. The basement membrane contains focal collections of collagen fibers within the lamina densa [62], a lesion that appears to be specific for NPS. The glomerular abnormality may progress to focal glomerular sclerosis, with tubular atrophy and interstitial fibrosis [29]. Immunofluorescence may show a nonspecific focal distribution of IgM or complement, especially if focal areas of sclerosis are present. We have observed specific ultrastructural changes in the kidneys of a 14-week spontaneously aborted fetus of a mother with the NPS, (figure 17-6), thus confirming the presence of the renal involvement during early intrauterine life.

Branchio-oto-renal syndrome (BOR)

The BOR syndrome is autosomal dominant and characterized by the association of branchial arch anomalies (preauricular pits, branchial fistulas), hearing loss, and renal hypoplasia and dysplasia [63, 64]. The syndrome occurs in about 2% of profoundly deaf children. Renal anomalies occur in 68% of

affected individuals [64]. It is estimated that severe renal dysplasia occurs in about 6% of heterozygotes. The presence of a preauricular pit at birth suggests that the child has at least one chance in 200 of severe hearing loss [65]. The renal anomalies range from minor deformities (sharply tapered superior poles, blunting of calyces) to marked hypoplasia, with renal failure in about 6% of patients.

Opitz–Frias syndrome (G or dysphagia–hypospadias syndrome)

This disorder was first described by Opitz et al. [66]. Clinical manifestations include swallowing problems with recurrent aspiration, stridorous respiration, intermittent pulmonary difficulty, weak hoarse cry, hypertelorism, slight slant of palpebral fissures [67], and hypospadies. Pathological studies have revealed laryngotracheal cleft malformations, tracheoesophageal fistulas, high carinae, pulmonary hypoplasia, cardiac defects, renal defects, imperforate anus, cryptorchidism, agenesis of gallbladder, and duodenal stricture [68]. A bifid renal pelvis and two ureters on the left side have been described in one case [69] and bilateral ureteral reflux during life in one other case. Autosomal dominant inheritance with partial sex limitation in expression seems the most likely form of inheritance.

Ectrodactyly–ectodermal dysplasia–cleft (EEC) syndrome

The association of ectrodactyly and cleft of lip and palate with ectodermal dysplasia was first described by Eckoldt and Martens [70] in 1804. Although most cases are isolated examples, there have been affected sibs [71–73] with normal parents, and several cases in which the disorder has been transmitted from a parent to one or more children [74]. Deafness, small or malformed auricles, and renal anomalies, particularly unilateral renal agenesis, may be present [73, 75–80]. Autosomal dominant inheritance with variable expression [76, 80] and even lack of penetrance has been noted in some families. However, the existence of a recessive form cannot be ruled out.

Townes–Brocks syndrome (thumb, auricular, anal and renal anomalies)

First described by Townes and Brocks [81], this autosomal dominant disorder includes thumb, auricular, anal, and renal anomalies [82, 83]. The syndrome has recently been extended to include congenital heart defects and anomalies of other internal organs [84]. It encompasses many of the anomalies of both the VATER association and the facio-auriculo-vertebral malformation sequence [85]. Renal anomalies include renal hypoplasia, ureterovesical reflux, and posterior urethral valves. Königsmonk and Gorlin [86] consider the syndrome due to an autosomal dominant trait.

Myotonic dystrophy and polycystic kidney disease

A family with myotonic dystrophy in which three siblings had cystic kidneys and the mother died of uremia was described by Emery et al. [87]. Te renal

abnormality is presumably the adult polycystic kidney inherited as an autosomal dominant trait. In addition to kidney lesions, abnormalities have included harelip, cleft palate, congenital heart disease, neurofibromatosis, spastic paraplegia, amyotrophic lateral sclerosis, mental retardation, deafness, epilepsy, limb malformations including syndactyly and talipes, eye defects such as microphthalmia, colobomata, and optic atrophy [87], and temporal blindness in males.

Ochoa syndrome

Elejalde [88] described seven patients (4 females and 3 males) in unrelated families, one with consanguinuity (first cousins–parents), who had a disorder characterized by peculiar facies, gestures while smiling and crying, hydronephrosis, hydroureter and intravesical stenosis of the ureter, abnormal caliber of the urethra in the prostatic and membranous portions, urethral valves, and a trabeculated bladder with diverticula, severe hypertrophy of the mucosa, and sclerotic changes. The condition is probably autosomal dominant, with variable expressivity and incomplete penetrance.

Brachmann–de Lange syndrome

Brachmann [89] and de Lange [90] described this syndrome in 1916 and 1933, respectively. Hundreds of cases are known. Abnormalities include short stature, retarded osseous maturation, mental retardation, microbrachycephaly, bushy eyebrows and synophrys, long curly eyelashes, small nose with anteverted nostrils, micrognathia, spurs on the anterior inferior rim of the mandible, hirsutism, micromelia of hands, arms, and feet, phocomelia and oligodactyly, clinodactyly of fifth fingers, simian crease, proximal implantation of thumbs, and flexion contractures at elbows. Choanal atresia, congenital heart defects, duplication of the gut, malrotation of the colon, brachyesophagus, pyloric stenosis, inguinal hernia, radial hypoplasia, short first metacarpal, and absent second to third interdigital triradius are sometimes present.

The kidneys have been described as hypoplastic, dysplastic, or cystic (figure 17-7). Few complete pathological descriptions have been reported. In the case of France et al. [91], the kidneys, which weighed only 5 g each, had numerous small cysts in the cortex and occasional foci of dysplasia, one of which contained a plaque of bone. The cause of the disease is unknown; most cases are sporadic. Familial occurrence is well documented, but the recurrence risk is low. In some cases, autosomal dominant inheritance seems likely.

Syndromes with amyloid nephropathy

Andrade syndrome (familial amyloidosis with polyneuropathy—type 1)

This familial form of amyloidosis is apparently inherited as an autosomal dominant trait occurring chiefly in the Portuguese and Japanese. A substitution of methionine for valine at position 30 has been found in these cases [92].

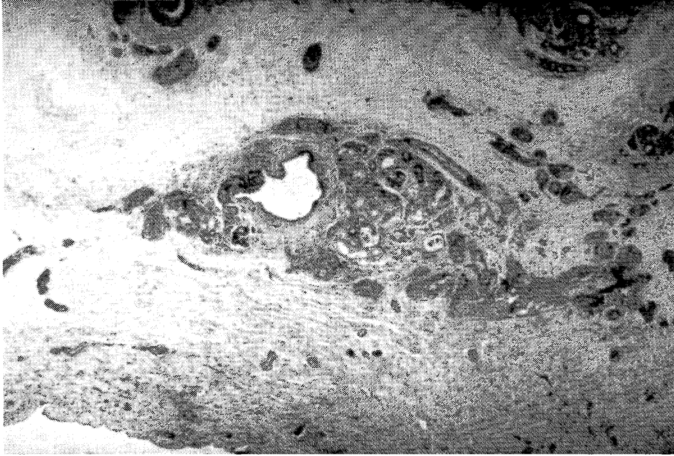


Figure 17-7. Hypoplastic dysplastic kidney in the Brachmann–de Lange syndrome.

Neuropathy involving the lower limbs and renal involvement with proteinuria occurs in some of the patients.

Van Allen syndrome (generalized amyloidosis with nephropathy, neuropathy, and peptic ulcer—type 2)

Van Allen et al. [93] described generalized amyloidosis in eight related persons, five in one generation and three in the next generation. Symptoms first appeared in the third and fourth decades. Average life span after recognition was 17 years. Consistent symptoms were nephropathy, neuropathy, and duodenal ulcer. Death was usually attributed to renal failure. Renal atrophy was the result of renal vascular amyloidosis. Dominant inheritance of a predisposition to amyloidosis is strongly suggested in this familial disorder.

AUTOSOMAL RECESSIVE DISORDERS

Renal lesions and associated abnormalities in autosomal recessive disorders are shown in table 17-2.

Meckel syndrome

The clinical and pathological characteristics of the Meckel syndrome (MS) were reviewed by Opitz and Howe [94], who suggested the eponym. The condition is recessively inherited and leads to death during the perinatal period or in early infancy; however, survival to 28 months has been reported [95]. In Finland, where there is a very high frequency of recessive disease, the probable incidence of MS was found to be 1:9000 births with equal sex ratio [96]. In other parts of the world, the incidence varies from 1:140,000 [97] to 1:13,250

Table 17-2. Autosomal recessive mutations

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Meckel syndrome	Adult type polycystic kidneys with occasional gigantic renal enlargement, vascular anomalies, hydronephrosis, cystic dysplastic kidneys and portal fibrosis, bilateral, diffuse cystic malformation of collecting tubules, agenesis, atresia of ureters, duplication of ureters, horseshoe kidney	Polydactyly, occipital encephalocele, CNS malformations, ocular anomalies, cleft palate, congenital heart defects, cysts of liver and pancreas
Goldson syndrome (Facio-auriculo-vertebral syndrome)	Cystic dysplasia	Defects of vermis of cerebellum, Dandy–Walker anomaly
Miranda syndrome	Cystic dysplasia with dysplasia of liver	Severe malformations of CNS, intrahepatic fibromuscular proliferation
Smith–Lemli–Opitz syndrome	Cystic dysplasia, malformation of renal artery, unilateral renal hypoplasia, nephrosclerosis, polycystic renal disease, perineoscrotal hypospadias, perineal urethral opening, cleft of scrotum, bilateral cryptorchidism in males, bifid pelvis, abnormality of the ureters, hydronephrosis, hypospadias, cryptorchidism	Mental retardation, microcephaly, hypotonia, incomplete development of external genitalia, minor anomalies of face, hands, and feet
Lissencephaly type II	Unilateral agenesis, cystic kidneys, micromulticystic kidneys	Obstructive hydrocephalus agyria and CNS abnormalities, Dandy–Walker malformation
Bardet–Biedl (BB) syndrome	Tubulointerstitial nephropathy with medullary cystic disease, occasional glomerular sclerosis, occasional cystic disease, dysplasia, caliectasis	Retinal pigment abnormality, obesity, mental retardation, hypogenitalism, polydactyly, syndactyly, anal atresia, anomalies of skull, congenital heart defects, deafness
Cockayne syndrome	Glomerular sclerosis, tubular atrophy, interstitial fibrosis, immune deposits, nephrotic syndrome	Microcephaly, senilelike changes, retinal degeneration, hearing defect, photosensitivity
Drash syndrome	Nephropathy (rapidly progressive glomerulonephritis and malignant nephrosclerosis), diffuse mesangial sclerosis, Wilms' tumor	Ambiguous genitalia, pseudohermaphroditism, streak gonads, gonadoblastoma
Johanson–Blizzard syndrome	Caliectasia, hydronephrosis, and single urogenital orifice	Aplasia nasal alae, hypothyroidism dwarfism, mental retardation, midline scalp defect, absent permanent teeth
Roberts syndrome	Horseshoe kidney and ureteral and single urogenital orifice	Phocomelialike limb deficiency, growth retardation, eye

Table 17-2 (continued)

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
		anomalies, hemangiomas, hypoplastic nasal cartilages
Winter syndrome (renal genital, and middle-ear anomalies)	Unilateral agenesis or hypoplasia, bilateral agenesis	Middle-ear anomalies, internal genital malformations
TAR syndrome (radial aplasia–Thrombocytopenia)	Unilateral renal agenesis, hypospadias, and transposition of penis and scrotum	Thrombocytropenia, leukemoid granulocytosis, anemia, limb defects, congenital heart defects
Hydrolethalus syndrome	Unilateral renal agenesis, hypoplasia of left kidney, cysts	Hydrocephalus, micrognathia, polydactyly, abnormal lobation of lungs, microphahalmia, cleft lip/palate, facial anomalies
Fryns syndrome	Kidney cysts	Facial and CNS anomalies, pulmonary hypoplasia, distal limb anomalies, cleft palate, diaphragmatic hernia
Oro-facial-digital syndrome (OFD) type I	Polycystic kidneys and liver	Webbing between buccal mucous membrane and alveolar ridge, partial clefts in the mid-upper lip, tongue, and alveolar ridges, dental abnormalities, hypoplasia of alar cartilages, asymmetric shortening of digits with clinodactyly, syndactyly, dry scalp, and variable mental deficiency
Osteochondrdysplasias		
Asphyxiating thoracic dystrophy	Tubulo-interstitial nephropathy with tubular dysfunction and progressive renal insufficiency in children surviving infancy; occasional dysplasia and diffuse cystic disease in newborns; frequent biliary dysgenesis	Contracted thorax, short limbs, biliary dysgenesis, portal fibrosis
Short-rib polydactyly (SRP) syndrome type I (Saldino–Noonan type)	Cystic dysplasia, hypoplasia of ureters	Dwarfism thoracic dystrophy, polydactyly, short limbs
Short-rib polydactyly (SRP) syndrome type II, (Majewski type)	Cystic dysplasia	Dwarfism, polydactyly, syndactly, cleft lip/palate, narrow thorax
Elejalde syndrome (Acrocephalo-polydactylous dysplasia)	Severe bilateral cystic dysplasia	Gigantism, polydactyly, acrocephaly, excessive connective tissue subcutaneous and visceral)
Peroxisomal Disorders		
Zellweger (cerebro-hepato-renal) syndrome (ZS)	Focal cortical, glomerular and tubular cysts, cystic dysplasia, altered metanephric duct	Growth retardation, characteristic facial appearance, hypotonia, congenital heart

Table 17-2 (continued)

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
	remnants, persistent fetal lobulations, horseshoe kidney, urethral duplication	defects, hepatic siderosis, cirrhosis, and CNS abnormalities
Hyperpipecolic acidemia	Renal tubular ectasia	Growth retardation, hypotonia, hepatomegaly, cirrhosis with glycogen storage, CNS abnormalities (protein and cerebellar demyelination)
Glutaric acidemia type II	Bilateral cystic kidneys, cystic dysplasia	"Sweaty feet" odor, CNS abnormalities, bile duct hypoplasia, cholestasis
Neonatally lethal adrenoleuko-dystrophy	Renal microcysts	CNS and adrenal abnormalities, retinal pigmentary degeneration anomalies, multiple congenital
Chondysplasia calcificans punctata, rhizomelic type	Micromulticystic kidneys with small glomerular anal tubular cysts	Symmetrical shortness of the humeri and femora, punctate epiphyseal calcifications, metaphyseal abnormalities, severe psychomotor retardation, microcephaly, cataracts

[98]. The classical triad includes polydactyly, occipital encephalocele (figure 17-8), and cystic kidneys. It is associated encephalocele (figure 17-8), and cystic kidneys. It is associated with other central nervous system malformations, cranial rachischisis, ocular anomalies, cleft palate, congenital heart disease, hypoplasia of the adrenal glands, pseudohermaphroditism in males, and other malformations. The renal lesions are variable, including hypoplastic kidneys, cystic changes resembling a mild form of adult polycystic kidneys, large cystic kidneys (figure 17-9) with vascular anomalies, hydronephrosis, dysplastic kidneys and misshapen kidneys (lack of lobulation and trilobed forms), agenesis, atresia, hypoplasia, and duplication of ureters and absence or hypoplasia of the urinary bladder [99]. Microscopically, the kidneys show cystic dysplasia (figure 17-10). There are sometimes cysts of the liver and pancreas.

In a study of 38 sibs with MS by Fraser and Lytwyn [16], all had cystic dysplasia of the kidneys, 63% had an occipital excephalocele, 55% had polydactyly, and 18% had no reported brain malformations. They concluded that the diagnosis of MS may not be valid in the absence of renal cystic dysplasia.

Difficulty may be encountered in differentiating very mild Meckel syndrome from rather severe Smith–Lemli–Opitz syndrome, the hydrolethalus syndrome, and trisomy 13.

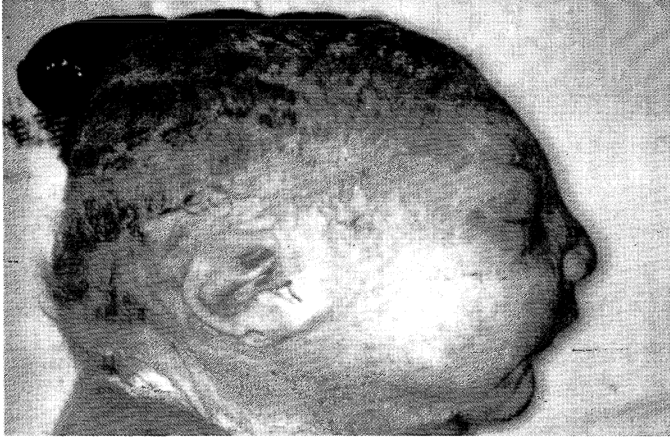


Figure 17-8. Meckel syndrome. Appearance of infant with encephalocele, sloping forehead and characteristic facial appearance.

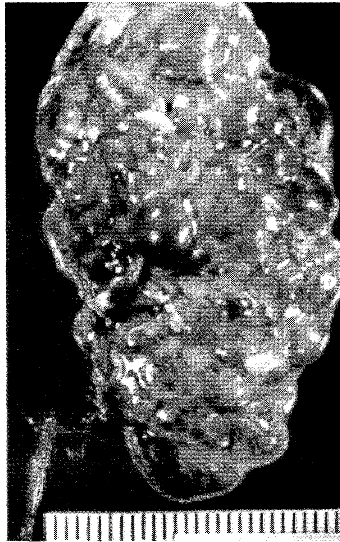


Figure 17-9. Large cystic kidneys in Meckel syndrome.

Goldstone and Miranda syndrome

Goldston et al. [100] described cystic dysplastic kidneys and abnormalities of the brain in three sibs. All had defects of the vermis and two had the Dandy–Walker anomaly. These infants did not have cystic changes in other organs.

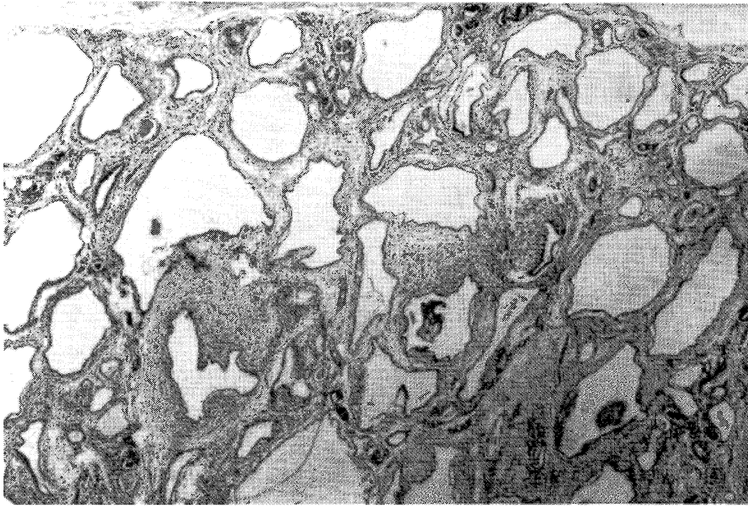


Figure 17-10. Microscopic appearance of kidney in Meckel syndrome showing cystic dysplasia. H&E Mag X100.

The kidneys contained innumerable tiny cysts and occasional larger cysts embedded in the fibrocellular stroma.

Two sibs with typical renal cystic dysplasia, severe malformations of the central nervous system, and diffuse intrahepatic fibroductular proliferation were described by Miranda et al. [101]. The brain lesions in one sib were occipital meningoencephalocele, dilatation of the fourth ventricle, dysplastic changes of the cerebellar vermis, internal hydrocephalus, and polymicrogyria of the cerebellum. The second infant had polymicrogyria and hydrocephalus due to apparent atresia of the Sylvian aqueduct. Occurrence in sibs in both these conditions suggests autosomal recessive inheritance. They are probably phenotypic variations of Meckel syndrome.

Smith–Lemli–Opitz syndrome

The Smith–Lemli–Opitz syndrome [102] is a common autosomal recessive malformation/mental retardation syndrome [94]. It is characterized clinically by microcephaly, mental retardation, growth failure, hypotonia, incomplete development of external genitalia (figure 17-11) in some affected males, and minor abnormalities of face, hands, and feet. Atypical mononuclear giant cells in pancreatic islets have been described [103]. Less frequent anomalies are rudimentary postaxial hexadactyly, congenital heart defect, and multiple anomalies of renal and spinal cord development. Thickening of basement membrances and hyalinization of the capillary tufts [104], stenotic and hypoplastic renal artery, cystic dysplastic kidneys, (figure 17-12), and hypoplastic

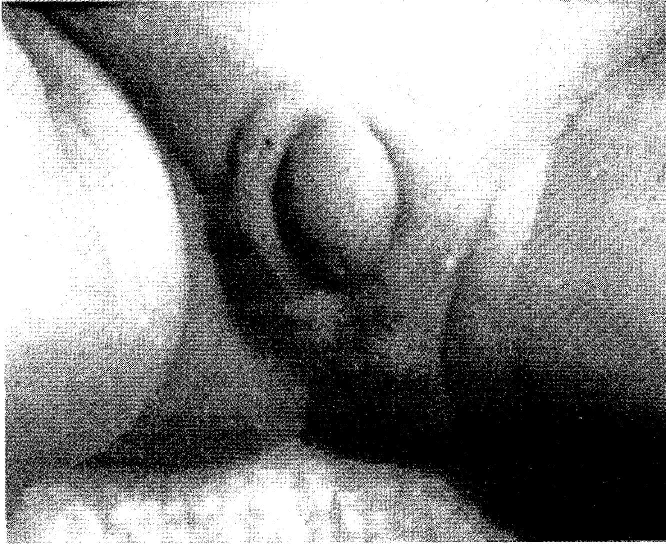


Figure 17-11. External genitalia in Smith–Lemli–Opitz syndrome.

ureters have also been encountered. Eight of the 13 cases reviewed by Cherstvoy et al. [105], which included previously reported cases, had anomalies of the genitourinary system (cystic renal disease, bifid pelvis, abnormalities of the ureters, hydronephrosis, hypospadias, and cryptorchidism). A few patients had severe hypospadias with perineal urethral opening, cleft of the scrotum, and bilateral cryptorchidism [40]. More than one sib has been affected in 40% of the families, and there are two recorded instances of parental consanguinity, suggesting autosomal recessive inheritance.

Lissencephaly type II (Walker–Warburg syndrome)

Type II lissencephaly is exemplified by the Walker–Warburg syndrome. The head and brain are larger due to obstructive hydrocephalus. The brain is globally agyric with a faintly pitted or verrucous surface. Coronal sections show a very thick gliovascular covering over the hemispheres and most other surfaces. The white matter is edematous and insufficiently myelinated. Heterotopic neurons are scattered widely with fewer large nests that do not protrude into the ventricular cavities. The brainstem and cerebellum are much more severely affected, and many patients have abnormalities consistent with a Dandy–Walker malformation and posterior encephalocele.

Agenesis of one kidney, cystic kidneys [111], cryptorchidism in males, and a small perineal body in females have been described. We have observed three patients with this syndrome associated with a mild Dandy–Walker like malformation; in two of these cases, the kidneys were micromulticystic.

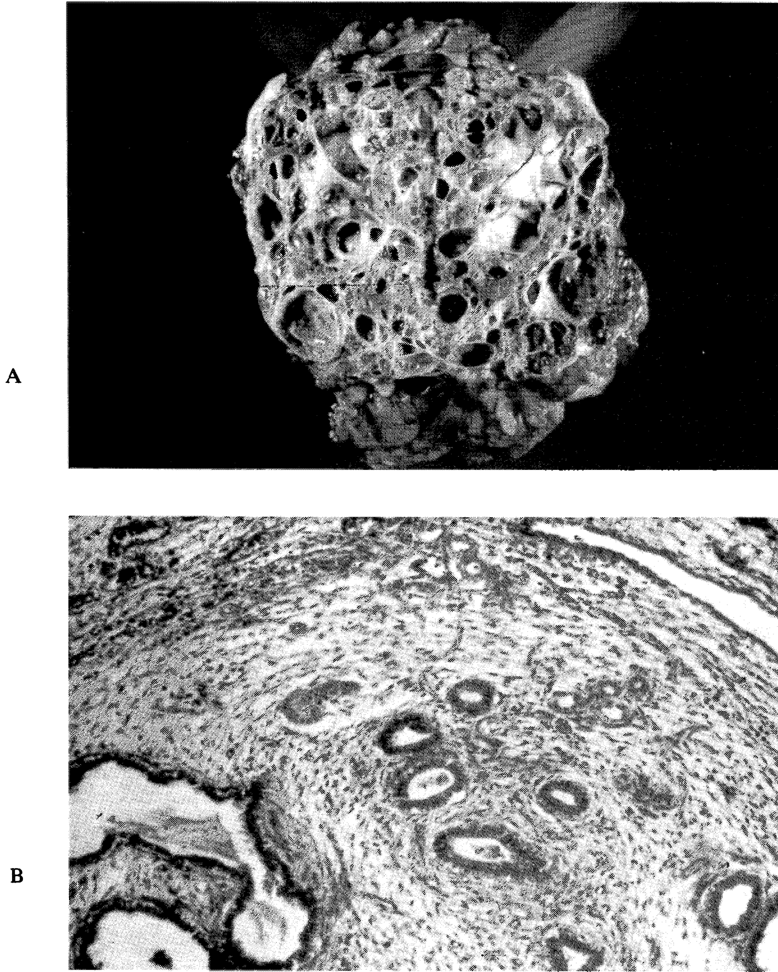


Figure 17-12. **A.** Large cystic kidneys. **B.** Microscopic section of the kidney in Smith–Lemli–Opitz syndrome showing cystic dysplasia with abundance of fibroconnective tissue marginating cystically dilated metanephric ducts. H&E Mag X100. (A and B, from: Gilbert EF, Opitz JM: Chapter 64, Renal Involvement in Genetic–Hereditary Malformation Syndromes. In: J. Hamburger J, Crosnier J, Grunfeld JP, (eds): Nephrology. New York: John Wiley & Sons, 1979, pp. 909–944.

Bardet–Biedel (BB) syndrome

Inherited as an autosomal recessive disorder, this syndrome includes retinal pigment abnormalities, usually in the form of retinitis pigmentosa, obesity, mental retardation, hypogenitalism, and polydactyly or syndactyly [111]. Less frequent anomalies are anal atresia, anomalies of the skull, congenital heart

defects, and deaf-mutism [112]. The paucity of autopsies in patients with this syndrome precludes an evaluation of possible renal malformations, although a high mortality from renal disease is known to occur. Glomerulonephritis, hydronephrosis, arteriolar nephrosclerosis, pyelonephritis, hypoplasia and congenital malformations of the pelvis, unilateral glomerular dysplasia [113], and tubulo-interstitial disease with medullary cyst formation resembling medullary cystic disease have been reported [114, 115].

Cockayne syndrome

Microcephaly and senile like changes beginning in infancy with retinal degeneration, impaired hearing, and photosensitivity of the skin were first described by Cockayne [77] in sibs. Since then, numerous cases have been reported [116]. Other abnormalities include growth failure with loss of adipose tissue, mental deficiency, muscle weakness with peripheral neuropathy, corneal opacities [117], cataracts, optic atrophy, deafness, and retinal blindness [116].

Renal abnormalities include glomerular sclerosis, tubular atrophy, and interstitial fibrosis [118]. Glomeruli contain deposits of immune globulin and podocytic alterations in association with proteinuria [119]. Progressive renal insufficiency may ensue.

Drash syndrome

The Drash syndrome is the triad of pseudohermaphroditism, nephropathy, and Wilms' tumor [120]. The renal lesions suggest rapidly progressive glomerulonephritis and malignant nephrosclerosis. Diffuse mesangial sclerosis, with progressive cortical atrophy, hemolytic uremic syndrome, segmental cortical necrosis (one case), and congenital nephrotic syndrome (two cases) have also been described.

One 46,XY phenotypic female had an unilateral ovotestis, streak gonad, and bilateral gonadoblastomas [121]. The coexistence of gonadal dysgenesis (46,XX) and elements of the Drash syndrome [122] supports the theory that there is a relationship between gonadal malformations, glomerulopathies, and an increased risk of renal neoplasia. Patients with acute renal failure and ambiguous genitalia should be evaluated carefully for this syndrome [121].

Johanson–Blizzard syndrome

Johanson and Blizzard [123] described three girls with congenital aplasia of the nasal alae, deafness, hypothyroidism, dwarfism, apparent metnal retardation, malabsorption, midline ectodermal scalp defects, and absent permanent teeth. Imperforate anus and urogenital malformations were inconsistently associated abnormalities. Other cases of trypsinogen deficiency [81, 124–127] are now recognized as examples of this syndrome. Johanson and Blizzard [123] also suggested that the patient reported by Grand et al. [128], with a 46,XXY chromosome constitution, pancreatic insufficiency, hypothyroidism, deafness, chronic lung disease, dwarfism, and microcephaly, may have had the same condition; however, photographs of his face are not convincing.

This syndrome is considered to be an autosomal recessive disorder. It incorporates elements of ectodermal dysplasia with endocrine and exocrine insufficiency, growth and mental retardation, and multiple congenital anomalies [123, 124, 127–130]. Renal anomalies include caliectasis, hydronephrosis, and a single urogenital orifice.

Roberts syndrome

This condition has been described as pseudothalidomide syndrome [131–134], Roberts phocomelia syndrome [135, 136], total phocomelia [137], hypomelia–hypotrichosis–facial hemangioma syndrome [138], and others [139].

The condition is a malformation syndrome that includes as the most prominent characteristics nearly symmetrical phocomelialike limb deficiency, prenatal and postnatal growth retardation, eye abnormalities including cloudy corneas, and minor craniofacial abnormalities such as silvery-blond hair, extensive hemangiomas, and hypoplastic nasal cartilages. Familial and sporadic cases have been reported. Premature centromere separation is a valuable laboratory diagnostic marker for this syndrome. This chromosome abnormality found in severe Roberts syndrome and the mildest of the so called SC syndrome strongly suggests that these two conditions are similar.

Autopsy studies have shown cystic dysplasia, horseshoe kidney, and ureterostenosis with hydronephrosis [140].

Winter syndrome (renal, genital, and middle-ear anomalies)

Winter et al. [141] described a familial syndrome of renal, genital, and middle-ear anomalies in four sisters who had renal hypoplasia or agenesis and internal genital malformations. Two of the children had malformations of the middle ear. One child had an unrelated chromosomal (47,XXX) anomaly.

The ovaries were represented by small nodules located below the adrenal glands. Peritoneal bands terminated in a small midline nodule representing the uterus. Two children had vaginal atresia. Embryological anomalies of the upper urinary system and the uterovaginal canal may be explained by interference with normal Wolffian duct development, since this structure is responsible for development of not only the ureteric bud and the metanephros, but also the Müllerian duct [142, 143].

The occurrence in two of these siblings of bilateral renal agenesis suggested a relationship to renal adysplasia, hereditary renal adysplasia, and unilateral renal agenesis. The vaginal atresia in the von Mayer–Rokitansky–Küster (MRK) malformation with renal anomalies appears to be related pathogenetically.

A second family with the Winter syndrome was reported by Turner [144].

TAR (thrombocytopenia absent radius) syndrome

Gross et al. [145] first described this entity in sibs; many other cases have subsequently been reported.

Hematologic abnormalities consist of thrombocytopenia with absence or hypoplasia of megakaryocytes, leukemoid granulocytosis (especially during

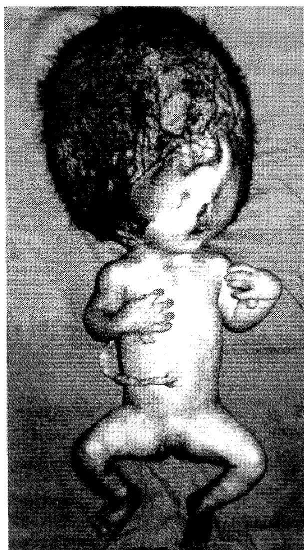


Figure 17-13. Clinical appearance of infant with hydrolethalus syndrome.

bleeding episodes), and frequently eosinosphilia and anemia. The limb defects include absence or hypoplasia of the radius, usually bilateral with associated ulnar hypoplasia and defects of the hands, legs, and feet.

Other abnormalities include congenital heart defects, spina bifida, brachycephaly, strabismus, micrognathia, syndactyly, short humerus, and dislocation of the hip. A genitourinary anomaly occurred in one of 40 cases reviewed by Hall et al. [146]; this patient had unilateral agenesis of the kidney with hypospadias and transposition of the penis and scrotum.

Hydrolethalus syndrome

Hydrolethalus syndrome was described by Salonen [147], who reviewed 28 cases. It includes hydrocephalus, micrognathia, polydactyly, and abnormal lobation of the lungs. Microphthalmia, cleft lip/palate, small tongue, anomalous nose, and low-set malformed ears are frequently present [148, 149] (figure 17-13). Unilateral hydronephrosis [150], bilateral pulmonary agenesis (BPA), unilateral renal agenesis hypoplasia of left kidney, and tubular cysts have been found [149]. We observed a hydrolethalus case (figure 17-13) delivered after decompression of the cranium by withdrawal of 3000 ml of cerebrospinal fluid. The kidneys were normal, but the adrenal glands were absent.

Fryns syndrome

The Fryns syndrome of CNS anomalies, microphthalmia, facial anomalies, pulmonary hypoplasia/abnormal lobation, diaphragmatic defects, urogenital

abnormalities, and distal limb anomalies was reported in a brother and sister who died in the newborn period [151]. They were discordant for cleft palate, diaphragmatic hernia, and Dandy–Walker anomaly. The overall pattern was similar to that seen in the cases of Fryns et al. [151] and Goddeeris et al. [152]. The case of Fitch et al. [154], a male born to second cousins, may represent a further example.

Microscopic examination of the kidneys uncovered the presence of numerous spherical cysts, mostly cortical, some of which were extremely large, the largest surrounded by fibrous and fibro-elastic connective tissue, consistent with cystic renal dysplasia. Some of the medullary areas showed marked fibrodysplasia and extremely immature mesenchymal elements. There were focal areas resembling minute Wilms' tumors.

Oro–facial–digital syndrome (OFD), Type 1

The OFD was identified as a syndrome in 1954, by Papillon-Leage and Psaume [154], although similar cases had been reported under a variety of names as early as 1860. The first complete description of the syndrome was made in 1962 by Gorlin and Psaume [155], and includes webbing between the buccal mucous membrane and the alveolar ridge, partial clefts in the mid-upper lip, tongue, and alveolar ridges, dental caries and anomalous anterior teeth, absent lateral incisors, hypoplasia of alar cartilages, asymmetric shortness of digits with clinodactyly, syndactyly, dry scalp, and variable degrees of mental deficiency [156, 157]. The report by Doege et al. [158] was based on three autopsies performed in one kindred. One infant had polycystic kidneys and another had polycystic kidneys and liver. Polycystic kidneys have also been observed in a mother and a daughter with this syndrome. Tucker et al. [159] subsequently described a newborn male with polycystic kidneys and liver. This case is now considered to be an example of Meckel syndrome. The condition is thought to be an X-linked dominant trait in heterozygous females, and a lethal trait in most hemizygous males.

Osteochondrdysplasias

Asphyxiating thoracic dysplasia (ATD) (Jeune syndrome)

Asphyxiating thoracic dystrophy is usually a lethal autosomal recessive disorder with constricted thorax, short limbs (rarely with polydactyly), and roentgenological abnormalities of the pelvis [160]. Yang et al. [161] described two types of ATD—type I and type II—based on the histologic pattern of endochondral ossification. They observed multiple renal cortical microcysts in type I. This baby also had congenital portal fibrosis.

The most common renal abnormality in children with ATD is tubulo-interstitial nephropathy, with a concentrating defect and impaired resorption of urate, phosphate, and amino acids [160]. The abnormality may progress to interstitial fibrosis and tubular atrophy, with secondary glomerular sclerosis [162] and renal insufficiency [160, 163]. This form of renal abnormality deve-

lops gradually in children who survive infancy, and it may be accompanied by hypertension. The presence of biliary dysgenesis and portal fibrosis, very much like the abnormalities of congenital hepatic fibrosis, may set the stage for portal hypertension and its sequelae in long survivors.

A few newborns and very young infants have a diffuse cystic disease or diffuse cystic dysplasia, with early onset of renal insufficiency. Although this variability may be interpreted as evidence of genetic heterogeneity, Bernstein et al. [26] interpreted the different morphologic patterns as resulting from the timing and severity of genetic expression. Early and severe expression of the heritable metabolic abnormality may alter renal morphogenesis. We also observed segmental dysplasia.

Short-rib polydactyly (SRP) syndrome type 1, Saldino–Noonan type

The Saldino–Noonan-type SRP syndrome, an autosomal recessive disorder, is characterized by thoracic dystrophy, postaxial hexadactyly, severely shortened, flipperlike limbs, and a striking metaphyseal dysplasia of the tubular bones [164]. Defective ossification is present in the calvarium, vertebral column, ribs, and short bones of the hands and feet. Cystic dysplastic kidneys were observed in one case and multiple renal cysts and hypoplastic ureters in another. In the third case the kidneys were normal [164]. In one case of Saldino and Noonan, the kidneys were described as "polycystic" [165].

Short-rib polydactyly (SRP) syndrome type 2, Majewski type

The Majewski-type SRP syndrome is a form of neonatal lethal dwarfism with short ribs and limbs, polydactyly, and syndactyly. It appears to be an autosomal recessive trait. Cleft lip and/or palate, pre- and postaxial polysyndactyly of all four limbs, and a short narrow thorax are frequent clinical manifestations [164]. Cystic changes in the kidneys have been described [166]. Infants with this syndrome die shortly after birth of severe respiratory distress. A patient we observed had severe bilateral cystic dysplasia of the kidneys [164].

Elejalde syndrome (acrocephalopolydactylous dysplasia)

Elejalde et al. [167] described an autosomal recessive disorder in a brother and sister, the last of 13 pregnancies including 3 spontaneous abortions and 8 normal children who were born to normal but consanguineous parents. The disorder was characterized by congenital gigantism, hexadactyly of the upper limbs in the male (figure 17-14), complete atypical premature fusion of all cranial sutures with acrocephaly, multiple minor anomalies of the face, nose, and auricles, and strikingly abnormal appearance of the entire fetus, with greatly increased thickness of the skin. Internal malformations included organomegaly and excessive amounts of connective tissue in all organs and tissues except for the CNS. The kidneys showed severe bilateral cystic dys-

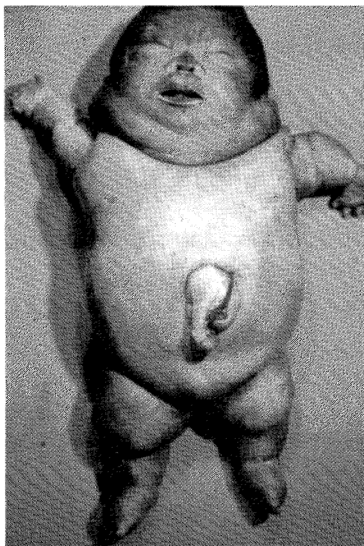


Figure 17-14. Elejalde syndrome. Clinical appearance of male infant showing gigantism and polydactyly.

plasia (figure 17-15). The changes have been considered to represent a hyperplastic dysplasia of mesodermal and ectodermal tissues.

Peroxisomal disorders

This is a group of disorders in which peroxisomal function is impaired. Some are associated with renal cysts and renal maldevelopments. The specific disorders are

Zellweger syndrome
Hyperpipecolic acidemia
Glutaric acidemia type II
Neonatal adrenoleukodystrophy
Adrenoleukodystrophy, adult type
Chondrodysplasia punctata, rhizomelic type

Zellweger (cerebro-hepato-renal) syndrome (ZS)

This disorder, which is due to an autosomal recessive trait, is lethal in infancy and dominated clinically by severe CNS dysfunction [168, 169]. Affected infants are generally born at term and do not manifest intrauterine growth retardation. They usually have a characteristic facial appearance, pear- or lightbulb-shaped head (figure 17-16), paucity of spontaneous movements, weakness and severe hypotonia with inability to suck, reduced deep tendon

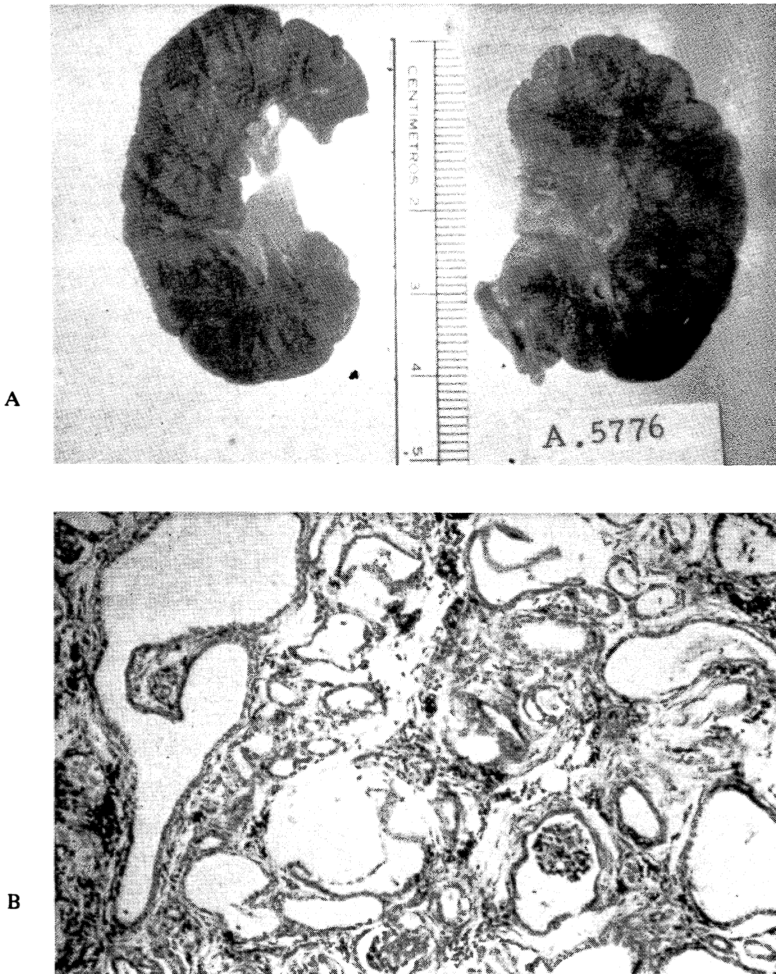


Figure 17-15. A. Gross appearance of kidneys in Elejalde syndrome. **B.** Microscopic section of kidney in Elejalde syndrome showing cystic dysplasia. H&E Mag X100. (A and B from: Elejalde BR, Giraldo C, Jimenez R, Gilbert EF: Acrocephalopolydactylous dysplasia. In: Bergsma D, Lowry RB (eds): New Syndromes. Alan R. Liss for the March of Dimes Birth Defects Foundation. Birth Defects 13(3B): 53–67, 1977.

reflexes, and total lack of psychomotor development [170–172]. Respiratory complications of hypotonia, with or without aspiration, are the usual cause of death before one year; the two oldest survivors lived for 14.5 and 15 months. Some atypical cases of the Zellweger syndrome (Versmold variant) may have hypertonia [173, 174]. Other manifestations include congenital heart defects (anomalies of aortic arch, patent ductus arteriosus, and ventricular septal defect), a skeletal lesion resembling chondrodysplasia calcificans punctata, and

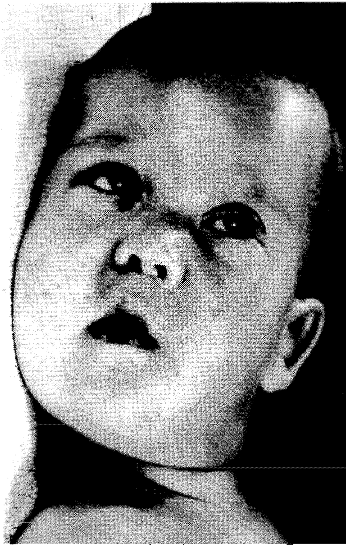


Figure 17-16. Zellweger syndrome. Characteristic facial appearance. (From: Gilchrist KW, Gilbert EF, Goldfarb S, Goli U, Spranger JW, Opitz JM: Studies of malformation syndromes of man XIB: The cerebro-hepatorenal syndrome of Zellweger: Comparative pathology. Eur J Pediatr 121:99–118, 1976.)

cirrhotic liver enlargement with signs of hepatic dysfunction (usually bleeding, less commonly jaundice). Increased serum iron content and evidence of tissue siderosis can be found in most cases before death and is helpful diagnostically [175]. Postmortem findings include increased brain weight (edema), focal lissencephaly and other cerebral gyral abnormalities, heterotopic cerebral cortex, olivary nuclear dysplasia, defects of the corpus callosum, the presence of numerous lipid-laden macrophages and histiocytes in cortical and periventricular areas, dysmyelination (probably of postnatal onset) [176], hepatic lobular disarray (micronodular cirrhosis), biliary dysgenesis, and persistence of renal fetal lobulations with cortical cysts (figure 17-17) [177]. Albuminuria and aminoaciduria have each been observed in about one fourth of the reported cases.

The cysts may be glomerular or tubular, and pericystic fibrosis with fibrous lamination around ductular structures suggests altered metanephric duct development (figure 17-18) [178]. Cysts have been more commonly glomerular in origin and occasionally have appeared to connect directly to terminal ends of collecting tubules without an intervening tubular segment, suggesting focally deficient metanephric differentiation. Many glomeruli, particularly in the outer third of the cortex, appear immature because of epithelial hyperplasia. Some glomeruli in the inner cortex show mild mesangial thickening that appears to progress to focal glomeulosclerosis. The renal cysts have been a constant finding in all cases reported and may be a consistent pathological

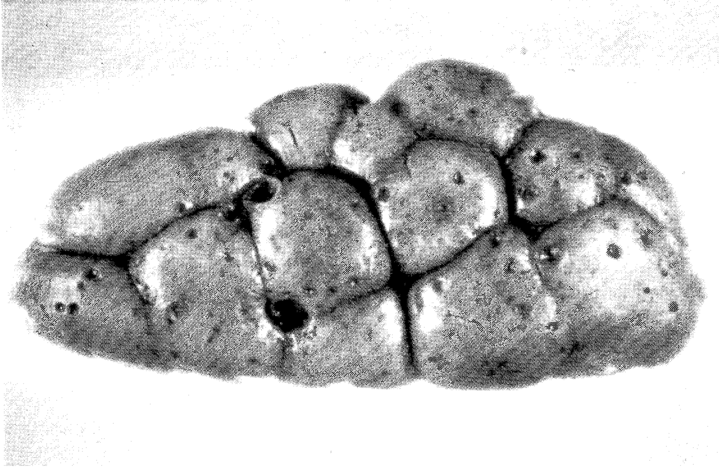


Figure 17-17. Gross appearance of kidney showing multiple small subcapsular cysts in Zellweger syndrome. (From: Gilchrist KW, Gilbert EF, Goldfarb S, Goli U, Spranger JW, Opitz JM: Studies of malformation syndromes of man XIB: The cerebro-hepatorenal syndrome of Zellweger: Comparative pathology. Eur J Pediatr 121:99–118, 1976.

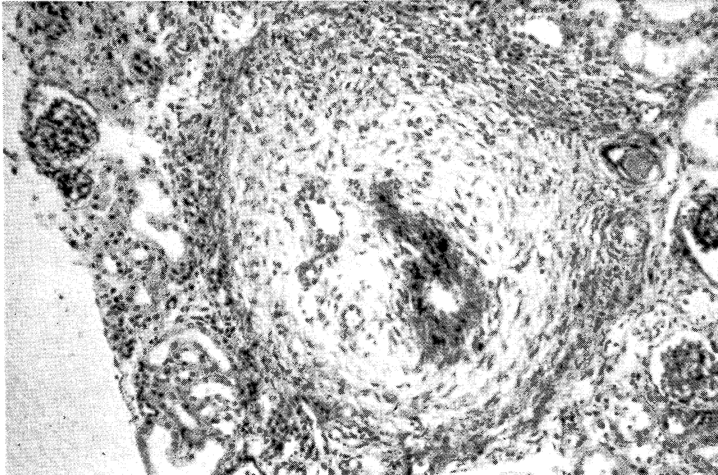


Figure 17-18. Microscopic appearance of pericystic fibrosis with fibrous lamination of ductular structures in Zellweger syndrome. H&E Mag X100. (From: Gilchrist KW, Gilbert EF, Goldfarb S, Goli U, Spranger JW, Opitz JM: Studies of malformation syndromes of man XIB: The cerebro-hepatorenal syndrome of Zellweger: Comparative pathology. Eur. J Pediatr 121:99–118, 1976.)

marker of this condition. Bernstein et a. [26] described in three infants, two of them siblings, a variant form of the ZS syndrome with renal cystic dysplastic changes. This apparently represents heterogeneity of the renal lesion since we observed similar dysplastic lesions in some of our cases. Horseshoe kidneys and ureteral duplication have also been noted [173]. Pulmonary hypoplasia, enlargement of islets of Langerhans, and cartilage abnormalities in the chondrodysplastic areas have been described. The diGeorge anomaly has been seen in several infants with the Zellweger syndrome.

The primary abnormality is a defective production of peroxisomal membrane proteins or of an enzyme required for import of peroxisomal proteins into these organelles [178]. It is presently unknown whether mitochondria are ultrastructurally abnormal because of the metabolic defect or vice versa. Reduced catalase activity correlates with apparent absence of peroxisomes. Since every cell in the body is metabolically defective, it is presumed that all congenital anomalies and subsequent pre- and postnatal organ and cellular structural changes represent a metabolic dysplasia sequence [161]. Absence of peroxisomes [179] seems responsible for defective oxidation of the cholesterol side chain during biosynthesis of cholic and chenodeoxycholic bile acids, leading to excretion of increased amounts of tri- and dihydroxycoprostanic acids [180, 181], and the defect in pipecolic acid metabolism [182]. Phosphatidyl ethanolamine plasmalogen is reduced in all cell membranes [178]. A defect in the electron transport chain before the step involving cytochromes [179, 183] is implicated.

A five-fold or greater increase of very-long-chain fatty acid levels, particularly hexacosanoic acid (C26:0) and hexacosenoic acid (C26:1), in plasma and cultured skin fibroblasts from 20 patients was demonstrated by Moser et al. [184]. Similar findings in cultured amniocytes from 3 of 14 women in whom the fetus was at risk of the Zellweger syndrome permitted prenatal diagnosis. Oxidation of very-long-chain fatty acids, which normally takes place in the peroxisome, was impaired in homogenates of cultured skin fibroblasts and amniocytes.

Hyperpipecolic acidemia

Four patients are known to have this Zellweger syndrome like metabolic defect [185–187]. It is characterized by failure to thrive, hypotonia, hepatomegaly, peripheral retinopathy, progressive neurological deterioration, simian creases, mongoloid slant of palpebral fissures, and death in the second year of life. Pathological changes include cirrhosis with glycogen storage, atrophic thymus, renal tubular ectasia, and pontine/cerebellar demyelination. The liver peroxisomes appear normal [185].

Glutaric acidemia type II

Glutaric acidemia type II is an inborn error of metabolism characterized by the accumulation and excretion of metabolites of the substrates of several acyl-

CoA dehydrogenases, including those specific for glutaryl-CoA, isovaleryl-CoA, and butyryl-CoA [188–195].

This disorder has been observed in six newborn infants [191, 194, 195]. Two other children [189, 193] and a 19-year-old woman [190] with similar metabolic and biochemical findings may represent milder variants. The severe form of the disease presents with overwhelming illness and leads to death within five days. As in two previously reported cases [194, 195], a "sweaty feet" odor has been noted. Hypoglycemia was not observed.

Two cases [192, 195] had Potter like characteristics and the kidneys were described as "polycystic." Two newborn brothers of Turkish origin had enlarged bilateral cystic kidneys, symmetric warty dysplasia of the cerebral cortex, and bile duct hypoplasia, cholestasis, siderosis, and fatty degeneration of the liver [196]. The renal medulla was completely cystic. The surrounding mesenchyme consisted of mature fibroblasts, loose collagen fibers, and a few hematopoietic foci. Cysts and dilated tubules were found scattered around the normal-appearing glomeruli and tubules. Renal papillae were hypoplastic and fibrocystic. We observed cystic changes in the kidney similar to those seen in the ZS, and it is therefore reasonable to consider this disease to be also a metabolic dysplasia [197, 198]. The basic defect is thought to be a deficiency of a cofactor or electron acceptor common to mitochondrial acyl-CoA dehydrogenases [197].

Neonatal adrenoleukodystrophy

Neonatal adrenoleukodystrophy (ALD) differs from childhood ALD with respect to age-of-onset, involvement of cerebral cortex, occurrence of pigmentary degeneration of the retina, the presence of multiple congenital anomalies, and pattern of inheritance (autosomal recessive). The neonatal form of ALD has never been observed in the same kindred as the childhood and adult form (an X-linked trait). All the patients have shown ultrastructural trilaminar lipid inclusions similar to those in childhood ALD, and all patients have had abnormally high levels of very-long-chain fatty acids in tissues and body fluids. Small renal cysts have been described by Jaffee et al. [199].

Absent peroxisomes and very unusual mitochondria have been noted, as in the ZS [184, 200]. Brown et al. [201] draw attention to the similarities between these two disorders and the accumulation of very-long-chain fatty acids in both conditions. Ultrastructural examination revealed that the inner adrenocortical cells contain lamellae and lamellar-lipid profiles of very-long-chain fatty acids-cholesterol esters, which are seen in the ZS as well [202].

Chondrodysplasia calcificans punctata, rhizomelic type

The rhizomelic form of chondrodysplasia punctata (figure 17-19) is an autosomal recessive trait characterized by symmetrical shortness of the humeri and femora, with punctate epiphyseal calcifications, metaphyseal abnormalities, severe psychomotor retardation, microcephaly, cataracts in over 70% of cases, and death usually during the first year of life. We observed micromulticystic

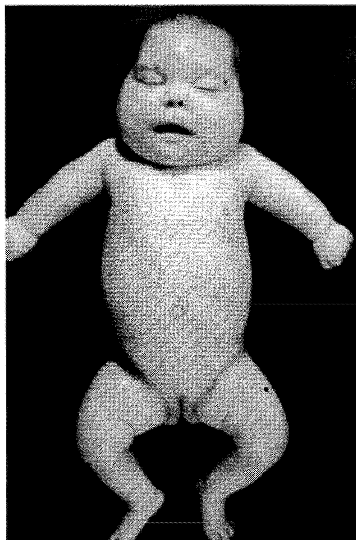


Figure 17-19. Chondrodysplasia punctata. Characteristic appearance of infant with "chipmunk"-like face and short-limbed dwarfism. (From: Gilbert EF, Opitz JM, Spranger JW, Langer LO Jr, Wolfson JJ, Viseskul C: Chondrodysplasia punctata—rhizomelic form: Pathologic and radiologic studies of three infants. Eur J Pediatr 123:89–109, 1976.

kidney lesions with small glomerular and tubular cysts (figure 17-20) [16]. No defects of renal function have been observed in this disorder.

Renal tubular dysgenesis

Renal tubular dysgenesis [203–205] is an unusual cause of neontal oliguria, usually after a gestation complicated by oligohydramnios. The kidneys are commonly although not necessarily enlarged, containing an increased number of nephrons [204], and the cortical tubules are lined with crowded columnar cells that have not differentiated to show the structural characteristics of proximal convoluted tubular epithelium. The glomeruli appear to be relatively crowded, therefore, and the medullary pyramids are smaller than normal. Recognition of this condition is important for family counseling, since it has been clearly shown to have an autosomal recessive inheritance [206].

X-Linked Recessive Disorders

Renal lesions and associated abnormalities in X-linked recessive disorders are shown in table 17-3.

Lowe syndrome (oculo-cerebro-renal syndrome)

Hypotonia, cataracts, renal tubular dysfunction, and mental retardation occurring as an X-linked disorder were first described by Lowe et al. [207]. The

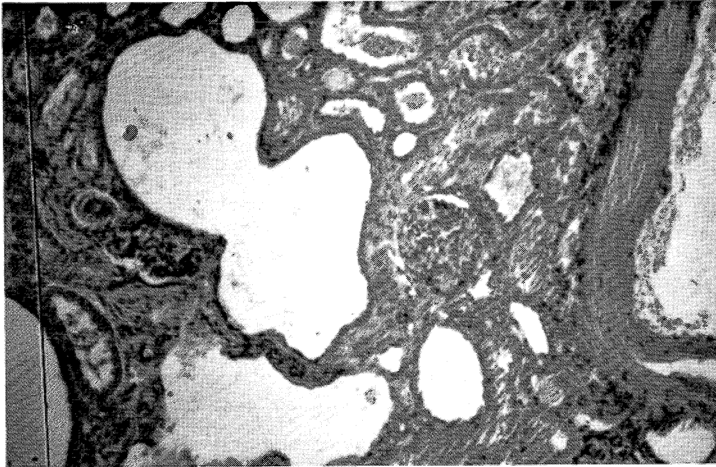


Figure 17-20. Microscopic section of kidney in rhizomelic chondrodysplasia punctata showing tubular and glomerular cysts. H&E Mag X100.

renal tubular defects consist of limited ammonium production, hyperchloremic acidosis, phosphaturia, hypophosphatemia, generalized aminoaciduria, albuminuria, osteoporosis, sometimes rickets, and organic aciduria [208, 209]. We have seen hypoplastic dysplastic kidneys in this disorder. Death is usually due to renal failure. It is inherited as an X-linked mutation.

Swyer syndrome (46, XXY gonadal dysgenesis) with real disease

The Swyer syndrome [210] is a Mendelian mutation (or series of mutations) that occurs in individuals who are either H-Y antigen positive or H-Y antigen negative. In spite of the fact that H-Y antigen may be present, the testes are not developed. Blanchet et al. [211] described a patient who, in addition to XY gonadal dysgenesis, had renal failure resulting from "interstitial nephritis" and who later developed a gonadoblastoma. Harkins et al. [212] also reported a patient with XY gonadal dysgenesis and renal failure. We observed renal failure in two patients with XY gonadal dysgenesis; one not only had gonadoblastoma but also a myotonic-dystrophy like condition. Excretory urography and cystoscopy showed no abnormalities, but proteinuria was present. Bilateral nephrectomy was performed at the time of renal transplantation. The kidneys were very small because of advanced glomerular sclerosis with marked tubular atrophy and interstitial fibrosis. The other child [213] had XY gonadal dysgenesis with gonadoblastoma and dysgerminoma in the dysgenetic gonads. A renal biopsy showed segmental glomerulosclerosis with immunofluorescence for complement component C3.

Four other similar cases have been reported, including concordance in a

Table 17-3. X-linked recessive mutations

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Oculo-cerebro-renal syndrome of Lowe	Renal tubular defect with limited ammonium production, hyperchloremic acidosis, phosphaturia, hypophosphatemia, generalized aminoaciduria, albuminuria	Mental retardation, glaucoma, cataracts, metabolic acidosis, organic aciduria, hypoplastic dysplastic kidneys
Oro-facial digital syndrome	Diffuse cystic disease, sometimes glomerulocystic disease	Webbing between buccal mucosa and alveolar ridge, clefts of lip, teeth anomalies, asymmetric short digits, hypoplasia of alar cartilages
Swyer syndrome (46,XY gonadal dysgenesis)	Glomerulosclerosis with tubular atrophy and interstitial fibrosis	Gondal dysgenesis, dysgenetic gonads, gonadoblastoma
Daentl syndrome	Duplication of left renal artery, disparity in size of kidneys, persistent fetal lobulations, lipid-laden cells in glomeruli, progressive focal glomerulo-sclerosis, nephrotic syndrome	Hydrocephalus, thin skin, blue sclerae, growth retardation, abnormal T-lymphocyte function
Goeminne syndrome (congenital muscular torticollis, multiple keloids, cryptorchidism, and renal dysplasia)	Renal dysplasia, chronic pyelonephritis with hypertension	Torticollis, multiple keloids, cryptorchidism, seminiferous tubule failure, multiple cutaneous nevi, varicose veins
Kallmann syndrome (congenial anosmia, hypogonadism, and unilateral renal agenesis)*	Unilateral renal agenesis	Congential anosmia, hypogonadism, cryptorchidism

*Some cases may be autosomal dominant with variable expression or autosomal recessive; genetic heterogeneity is likely.

pair of monozygotic twins. Because of the risk of gonadal malignancy, the karyotype and serum FSH concentration should be determined in phenotypic females with primary amenorrhea and chronic renal disease, and gonadectomy should be performed in all cases of XY gonadal dysgenesis.

Daentl syndrome

The concurrence of nephrosis, hydrocephalus, thin skin, blue sclerae, growth retardation, abnormal T-lymphocyte function, and a distinctive face was reported in two borthers [214]. Preliminary studies suggest that at least some of the clinical findings may be the result of a disorder of collagen synthesis. The disease is lethal.

In one case both kidneys were small and pale, with global sclerosis of essentially all glomeruli: extensive areas of tubular atrophy, interstitial fibrosis, and severe vascular sclerosis. In the other case, the size of the two kidneys was markedly different, 60 g vs. 10 g. Fetal lobulation was present. The left renal artery was duplicated; however, neither branch appeared stenotic. Glomeruli of the outer cortical areas contained lipid-laden cells. Deeper glomeruli were hyalinized. In both affected brothers, the renal changes were suggestive of progressive focal glomerulosclerosis. The blue appearance of the sclerae was caused by thinning of the scleral coat. An unexpected finding was the presence of extensive longitudinal plaques in the aorta. Skin fibroblast cultures have shown a defect of type III procollagen.

Goeminne syndrome (congenital muscular torticollis, multiple keloids, cryptorchidism, and renal dysplasia)

A probable X-linked syndrome of congenital muscular torticollis, multiple keloids, cryptorchidism, and renal dysplasia was described by Goemionne [215]. Additional manifestations are seminiferous tubule failure with normal Leydig cell function, multiple cutaneous nevi, and varicose veins. The renal lesion progresses to a chronic pyelonephritis with hypertension. The symptoms appear early in youth. Males are more severely affected than females.

Kallmann syndrome (congenital anosmia, hypogonadism, and unilateral renal agenesis)

Familial occurrence of congenital anosmia and hypogonadism was first reported by Kallmann et al. [216]. Nowakowski and Lenz [217] suggested an X-linked inheritance in one family with anosmic hypogonadotropic hypogonadism (AHH); they observed unilateral renal agenesis in one of two affected half-brothers from a second family. Sparkes et al. [218] detected the syndrome in two males and their half-sister, and also postulated X-linked transmission. Eight males were affected in another kindred [219, 220]; many were cryptorchid, and one had unilateral renal agenesis. X-linked AHH with unilateral renal agenesis was reported by Wegenke et al. [221] and Lueteneggcr et al. [222]. Other reports ae consistent with autosomal dominant inheritance with variable expression [223–226]. Analysis of other reported families [227–232] suggests autosomal recessive inheritance. Genetic heterogeneity of AHH is likely, since both male-to-male transmission and X-linked pedigree patterns have been reported.

Unilateral renal agenesis was documented in another possible sporadic case [233]. This case, however, may represent either the Kallmann syndrome or the LEOPARD syndrome.

USUALLY SPORADIC DISORDERS

Renal lesions and associated abnormalities in condictions that are usually sporadic are shown in table 17-4.

Table 17-4. Usually sporadic mutations

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Goldenhar complex (Facio-auriculo-vertebral syndrome)	Pelvic deformity, anomalous renal artery, unilateral cystic kidney	Microtia, preauricular tags, deafness, vertebral anomalies
Klippel–Trenaunay–Weber dysplasia	Diffuse bilateral nephroblastomatosis	Unilateral limb hypertrophy, cutaneous hemangiomas, varicose veins, osseus and soft tissue hypertrophy, thrombocytopenia, visceral angiomatosis
Wiedemann–Beckwith (WB)* syndrome	Enlarged kidneys, persistent glomerulogenesis, diffuse bilateral nephroblastomastosis, metanephric hamartomas, hydronephrosis and hydroureters, Wilms' tumor, duplication of collecting system, dysmorphogenetic kidneys, disorganized renal parenchyma with fissures and abnormal lobulations, corticomedullary disarray	Macrosomia, macroglossia, hypoglycemia, omphalocele
Williams syndrome	Renal artery stenosis, degenerative renal disease, small penis	Mental retardation, supravalvular aortic stenosis, partial anodontia
Rubinstein–Taybi syndrome	Duplication of kidneys and ureter, absence of kidney, hydronephrosis, abnormality of bladder shape, bladder diverticulum, posterior urethral valves	Mental and motor retardation, broad terminal phalanges of thumbs and great toes, short stature, characteristic facial appearance
Russell–Silver syndrome	Bilateral chronic pyelonephritis, urethral pelvic obstruction with severe reflux	Short stature, hemihypertrophy, elevated urinary gonadotropins, craniofacial dysostosis
*Urinary Obstruction Sequences*** (genetics depends on etiology)	Obstructive lesions	Oligohydramnios, Potter phenotype, pulmonary hypoplasia
Prune belly sequence*** and related defects	Developmental dysplasia of smooth muscle of urinary tract, hydroureters and hydronephrosis, urethral or bladder neck obstruction, renal dysplasia, megalourethra, megacystis, megaureters, renal hypoplasia, salt-losing nephritis	
Associations		
VATER association and variants	Renal dysplasia or agenesis, persistent urachus, renal ectopia, hypospadias, caudally displaced dysplastic penis,	Vertebral and cardiac anomalies, traceoesophageal fistula, anal stenosis, radial dysplasia

Table 17-4 (continued)

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
	ureterovesical reflux, ureteropelvic obstruction, cross-fused ectopia	
MURCS association	Renal agenesis or ectopy, absence of both kidneys, ureters and renal arteries, renal aplasia	Vertebral defects, absent vagina and uterus
CHARGE association	Duplicated upper pole of one kidney, hydronephrosis, unilateral renal agenesis	Coloboma, choanal atresia, cardiac, genital, and ear defects
Schisis association	Defects of urinary tract, renal agenesis	Neural tube defects, oral clefts, omphalocele, diaphragmatic hernia, cardiac defects, limb deficiencies

*Usually sporadic; familial cases, delayed mutation of an unstable premutated autosomal dominant gene.
**Familial occurrence of postior urethral valves has been reported.
***Inheritance unknown; ? unstable autosomal dominant or X-linked premutation.

Goldenhar complex (facio-auriculo-vertebral "syndrome")

This seems to be a complex malformation affecting the first arch and vertebral column in association with anomalies of viscera [234]. Ear anomalies are common (figure 17-21). The kidneys were not remarkable in one case described by Opitz and Faith [235], but abnormalities of the collecting system were described by Gorlin et al. [234]. Gross [236] reported an anomalous blood supply to one kidney. Caramia et al. [237] described a case with cardiovascular malformations, agenesis of the right lung, situs inversus, and cystic kidney. Familial occurrence has not been reported.

Klippel–Trenaunay–Weber dysplasia

The triad of cutaneous hemangioma, varicose veins, and osseous and soft tissue hypertrophy was first described in 1900 by Klippel and Trenaunay [238]. Parkes-Weber [239] described a similar condition associated with arteriovenous fistulae. Visceral angiomatosis is uncommon [240, 241]. Hemangiomata of the urinary bladder and glans penis may rarely occur [242]. Thrombocytopenia is frequently present [243].

Mankad et al. [244] observed diffuse bilateral nephroblastomatosis in a case with cutaneous hemangiomata and bone and soft tissue hypertrophy of the limbs [40]. Cysts of the kidney were not observed [40]. However, nephroblasmatosis, as it regressess or matures under treatment and sometimes spontaneousy, may become cystic.

Wiedemann–Beckwith (WB) syndrome

The WB syndrome of exomphalos, macroglossia, and gigantism, described by Wiedemann [245] and later by Beckwith [246], includes neonatal hypogly-

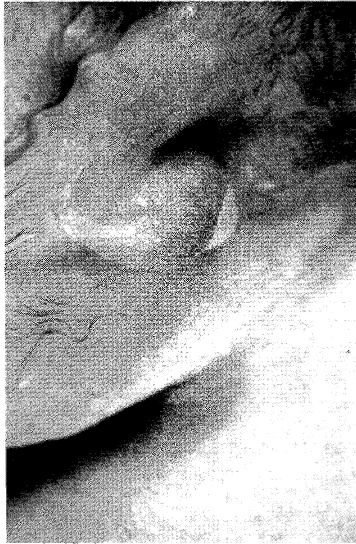


Figure 17-21. Ear anomaly in the Goldenhar syndrome. Microtia with rudimentary auricle and absence of external auditory meatus.

cemia, organomegaly, cytomegaly of the adrenal cortex and islet cells of the pancreas, and a predisposition to the development of malignant tumors including Wilms' tumor, adrenocortical carcinoma, hepatoblastoma, gonadoblastoma, and brainstem glioma [247]. The renal changes include enlarged kidneys, persistent glomerulogenesis, medullary dysplasia, diffuse bilateral nephroblastomatosis, metanephric hamartomas, hydronephrosis and hydroureters, and duplications [247]. The kidneys are enlarged and noncystic. Their surfaces are traversed by numerous irregularly disposed shallow fissures, increasing the number of lobulations [246]. Sections show a disorganized parenchyma with minute lobulations crowding one another, each with a distinctly demarcated cortex and medulla, with many abnormally pale medullary pyramids. There is no pelvic dilatation. The primary branches of the calyceal system are apparently increased in number, but secondary branches are clearly definable, producing a complex arborization of the collecting system. Most pyramids have an increased proportion of stroma, with immature collecting tubules characteristic of medually dysplasia. We observed similar dysmorphogenetic changes and corticomedullary disarray (figure 17–22). Wilms' tumor associated with this syndrome may be bilateral.

Most cases have been sporadic, although occasional familial cases have been reported. A delayed mutation of an unstable premutated gene has been postulated [247]. In other families this is a dominantly inherited condition.

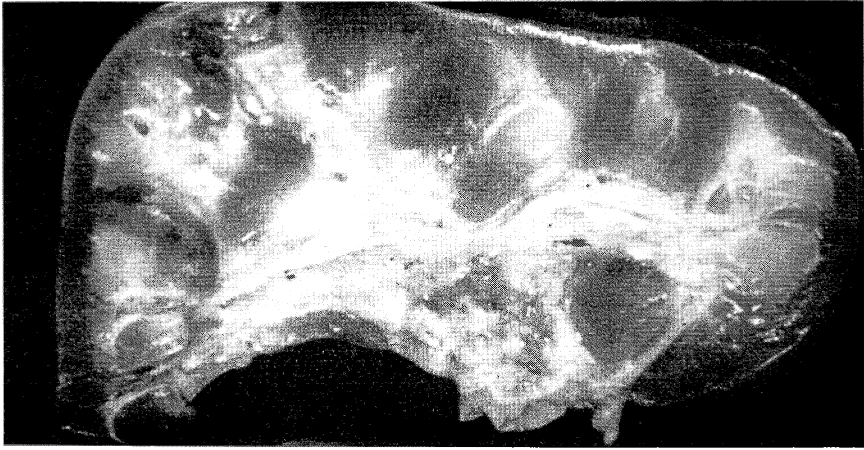


Figure 17-22. Gross appearance of cut surface of dysmorphogenetic kidney.

Williams syndrome

Williams syndrome is characterized by mental deficiency, an elfin facial appearance with medial eyebrow flare, short palpebral fissures, depressed nasal bridge, epicanthal folds, periorbital fullness of subcutaneous tissues, and supravalvular aortic stenosis [248, 249]. Genitourinary abnormalities include bladder diverticula [250], small penis, and degenerative renal disease. Peripheral pulmonary artery stenosis, pulmonic valvular stenosis, ventricular and atrial septal defects, renal artery stenosis and hypertension, hypoplasia of the aorta and other arterial anomalies may occur.

There is evidence, both from clinical observation of cases of neonatal hypercalcemia and from experimental studies of hypervitaminosis D in pregnant rabbits [251–253], that all manifestations seen in infants with the Williams syndrome are due to fetal hypercalcemia and its consequences, even though postnatal hypercalcemia is infrequent.

The syndrome is usually sporadic with negligibly small empiric recurrence risk; two patients who were second cousins have been reported [254].

Rubinstein–Taybi syndrome

Rubinstein and Taybi in 1963 [255] described seven patients with a syndrome characterized by mental and motor retardation, broad terminal phalanges of thumbs and great toes, short stature, small head, characteristic facial appearance with beaked nose, antimongoloid slant of palpebral fissures, highly arched palate, and cryptorchidism in males. Anomalies of the urinary tract occur in 50% of cases and include duplicated kidney and ureter [256], "absence" of a kidney or absent renal function on one side [257, 258], pyelonephritis

[256–258], nephrolithiasis [256], abnormality of bladder shape [259], bladder diverticulum [260], hydronephrosis with or without posterior urethral valves [261, 262], and unilateral hydroureter [263]. This disorder appears to be sporadic [264]. It has occurred in one of monozygotic twins [257] and in one of dizygotic female twins [265].

Russell–Silver syndrome

Silver et al. [266] described two children with short stature, hemihypertrophy, and elevated urinary gonadotropins. Later, Russell [267] reported five patients with intrauterine growth retardation, craniofacial dysostosis, and disproportionately short arms [267]. Abnormal excretory urograms and cystograms, including bilateral chronic pyelonephritis, unilateral ureteral–pelvic obstruction with severe reflux, and unilateral pyelonephritis were found [268].

Since these are potentially treatable anomalies, patients with Russell–Silver syndrome should have renal ultrasonography and cystogram. The condition is usually sporadic; however, autosomal dominant inheritance has been suggested in some cases.

Urinary obstruction sequences

Early urethral obstruction sequence—cystic dysplasia (Potter type IV)

Early urethral obstruction is commonly the consequence of posterior urethral valves during the development of the prostatic urethra [269–271], resulting in dilatation of the renal tubules and the development of renal cysts [33, 272]. The condition is sporadic, but familial occurrence of posterior urethral valves has been recorded.

Prune belly sequence and related defects

First reported by Parker [273] but later fully described by Eagle and Barrett [274] in nine children, this condition is a triad of apparent absence of abdominal muscles, urinary tract defects, and cryptorchidism. Cephalad displacement of the umbilicus, flared rib margins, Harrison's groove, and pectus deformities are all probably secondary to the muscle defect. There is a frequent association with talipes equinovarus.

The disorder is thought to be due to an intrauterine obstruction of the proximal urethra leading to urinary tract and abdominal distention. Burton and Dillard [275] speculate that this defect occurs as a result of splitting of the abdominal wall secondary to massive bladder dilatation and stretching of the abdominal muscles. A generalized attenuation of recognizable smooth muscle elements, with lack of differentiation into circular and longitudinal orientation of smooth muscle fibers, and profound replacement with collagen tissue has been described [276]. Renal dysplasia is present in those cases in which the ureters had a large amount of fibrous collagen deposition. The urinary tract is greatly dilated with a high incidence of urethral or bladder neck obstruction. The clinical course varies from neonatal death (20% of infants) to long-term

survival without evidence of significant renal impairment. Megalourethra has been noted [277], as well as dilatation of the prostatic urethra (presumably due to lack of muscular support of the prostate), megacystis, megaureters, renal hypoplasia, and hydronephrosis. Decreased spermatogenesis and absence of spermatogonia have also been described [278]. Salt-losing nephritis has been observed [279]. Atony of the bladder and ureters makes urinary stasis a constant clinical manifestation. Intraluminal pressures are not high, and pressure-induced changes in renal function are not a major problem. Preservation of infection-free urine helps maintain renal function.

Potter oligohydramnios sequence

Urinary tract defects with lack of urine formation in utero result in lack of amniotic fluid. The primary defects include bilateral renal agenesis, aplasia, severe hypoplasia and dysplasia, infantile polycystic kidneys, and urinary tract obstruction. Chronic leakage of amniotic fluid in midgestation may also lead to oligohydramnios. The secondary effects are fetal compression with the typical Potter appearance (figure 17-22) [280]. The cause of death is respiratory failure due to lack of late alveolar development [281].

ASSOCIATIONS

VATER association and variants

The term *VATER association* was suggested by Quan and Smith [282] for a nonrandom occurrence of anomalies, including vertebral defects, anal atresia, tracheoesophageal fistula with esophageal atresia, and radial and renal defects. Other frequent malformations included in this association are ventricular septal defects, single umbilical artery, duodenal atresia, auricular defects, cleft lip and palate, absence of spleen, unilateral aplasia of lung or testis, female pseudohermaphroditism, and unilateral hypoplasia of hip bone and common iliac artery. VACTERL is one of many expansions used to include cardiac and limb defects. In a recent review of tracheal agenesis and associated malformations [283] and in an editorial comment by Lubinsky [284], the spectrum of the VACTERL association was found to overlap caudally with the caudal regression anomaly and MURCS association (see below) [285], and cephalically with tracheal agenesis and the hemifacial microsomia and other facial asymmetry syndromes [286, 287]. All may be different manifestations of a broader axial mesodermal dysplasia spectrum [288] or may represent different underlying mesodermal defects that may occur separately or concurrently in different individuals. The genitourinary defects include renal dysplasia or agenesis, renal ectopia, persistent urachus, hypospadias, and caudally displaced dysplastic penis.

Genitourinary abnormalities are common in the VATER association [278], although they might be overlooked in very young infants because of other, life-threatening anomalies. Further, the genitourinary abnormalities are often

persistent and may be important to the long-term outcome of the patient. Of 23 patients, 21 had renal or urologic anomalies: 9 had ureterovesical reflux, 7 renal agenesis, 5 hydronephrosis secondary to ureteropelvic junction obstruction, and 5 crossed fused ectopia. Embryologically, the VATER association seems to originate in a disturbance occurring before the 35th day of gestation [282]. Most cases are sporadic, without evidence of genetic or proven environmental factors. The same malformations occur in other disorders, including chromosome aberrations. Lubinsky [289] has hypothesized that all associations represent disruption sequences—hence, the low empiric recurrence risk after birth of one affected child. A common metabolic disurptive condition is maternal diabetes in infants with the VATER association. Dominant inheritance of some of the components of the VATER association has been reported [81, 290, 291].

MURCS association

MURCS is an acronym for Mullerian duct aplasia, renal aplasia, and cervicothoracic somite malformation. This nonrandom association includes cervicothoracic vertebral defects, especially from C5 to T1 (sometimes termed the Klippel–Feil malformation sequence [292, 293], absence of the vagina [294], absence or hypoplasia of the uterus, and renal abnormalities. The latter include renal agensis and ectopy, and absence of both kidneys, ureters, and renal arteries [292].

CHARGE association

The CHARGE association shows some phenotypic overlap with the VATER association. CHARGE is an acronym for coloboma, heart disease, atresia choanae, and retarded growth and development. Associated anomalies are genital and ear anomalies, tracheoesophageal fistula, facial palsy, micrognathia, cleft lip, cleft palate, and omphalocele. Anomalies simulating trisomy 18, trisomy 13, del(4p), and cat-eye syndrome have been described [85, 295]. Renal anomalies include duplicated upper pole of one kidney and hydronephrosis, and unilateral renal agenesis [296, 297].

Many of the anomalies present in the CHARGE association may represent arrested development between days 35 and 45 of gestation [85].

Familial occurrence of some of the associated anomalies has suggested a possible genetic cause [298]. In the case of normal parents of an affected child, there appears to be a low but not negligible recurrence risk [85].

Schisis association and its variants

Schisis (midline) defects, including neural tube defects (anencephaly, encephalocele, spina bifida cystica), oral clefts (cleft lip and palate, posterior cleft palate), omphalocele, and diaphragmatic hernia associate with one another far more frequently than at the expected random combination rates. The schisis association is practically a lethal abnormality. It occurs more often in girls, in

twins (4.6%), in breech presentations (13.7%), in association with lower birth weight, and in association with a shorter gestation.

Congenital cardiac defects, limb deficiencies, and defects of the urinary tract, mainly renal agenesis, have a high correlation value [299].

Opitz and Gilbert [300] relate these defects to the midline developmental field, the morphogenetic properties of which are particularly poorly buffered.

Several pleiotropic mutations seem to exert their effect predominantly on the midline; for example, G (hypospadias–dysphagia) syndrome [301] and the recessive hydrolethalus syndrome and many midline anomalies suggest generalized midline weakness. If midline development is less buffered than that of paramedian structures, then aneuploid individuals ought to have a greater liability to midline defects than nonaneuploid individuals [300, 302–305].

TERATOGENIC ABNORMALITIES (SECONDARY MALFORMATIONS, DISRUPTION SEQUENCES)

Renal lesions and associated abnormalities in teratogenic abnormalities are shown in table 17-5.

Fetal alcohol syndrome

The fetal alcohol syndrome was first described by Lemoine et al. [306], and later by Jones and Smith [248], in the offspring of chronically alcoholic women. Alcohol is now considered the most common major teratogen to which the fetus is exposed [307]. It is the most common cause of intrauterine growth retardation and congenital anomalies among North American infants. Some 10%–20% of all Indian infants may be affected.

Variable manifestations include pre- and postnatal growth deficiency, mental retardation, mild to moderate microcephaly, short palpebral fissures, maxil-

Table 17-5. Teratogenic abnormalities

Condition	Abnormalities of kidney and urinary tract	Associated abnormalities
Fetal alcohol syndrome	Small rotated kidneys, hydronephrosis	Intrauterine growth retardation, microcephaly, short palpebral fissures, typical phenotype, limb defects, congenital cardiac defects
Diabetic embryopathy	Renal agenesis and renal dysplasia	Brain, heart, and skeletal anomalies, caudial regression syndrome
Warfarin embryopathy	Unilateral renal agenesis, abnormal urinary tract	Nasal hypoplasia, stippled epiplyses, CNS and eye abnormalities
Thalidomide embryopathy	Renal agenesis, hypoplasia, hydronephrosis, horseshoe kidney, cystic kidneys, renal ectopia, anomalies of rotation	Limb deficiency—phocomelia, heart, intestinal eye and ear anomalies, genitourinary anomalies

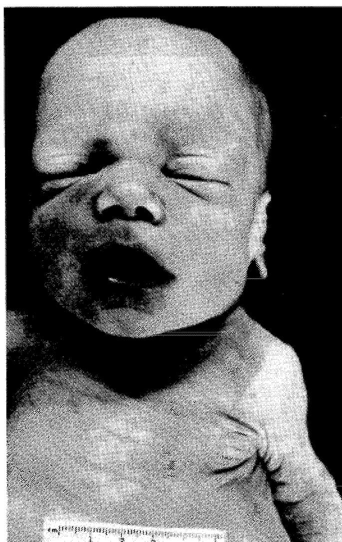


Figure 17-23. Typical facial appearance of the Potter sequence.

lary hypoplasia, short nose, smooth philtrum with thin and smooth upper lip vermilion, cardiac defects, and more or less severe limb defects [308–312] (figure 17-23). Renal anomalies in 10%–20% of cases include hypoplasia, malrotation, and hydronephrosis [313].

Diabetic embryopathy

Maternal diabetes resulting in metabolic derangement is capable of causing developmental defects such as brain anomalies, congenital heart disease, spina bifida, sirenomelia, imperforate anus, and radius aplasia [314–320]. Some of these children are designated as having the "caudal regression syndrome" (figure 17-24), which is a double misnomer since it is neither a syndrome nor a regression of tissues present earlier, as in other children having the VATER association. Renal anomalies are similar to those seen in sirenomelia and include renal agenesis and renal dysplasia [321–323].

Warfarin embryopathy

Administration of coumarin derivatives to women during the first trimester of pregnancy causes a specific constellation of malformations known as the warfarin embryopathy or fetal warfarin syndrome [324–330]. The most constant manifestations are nasal hypoplasia and stippled epiphyses; others are central nervous system and eye abnormalities [331].

In a review by Hall et al. [331] of 24 patients, two had unilateral renal agenesis [332] and abnormalities of the urinary tract [330].

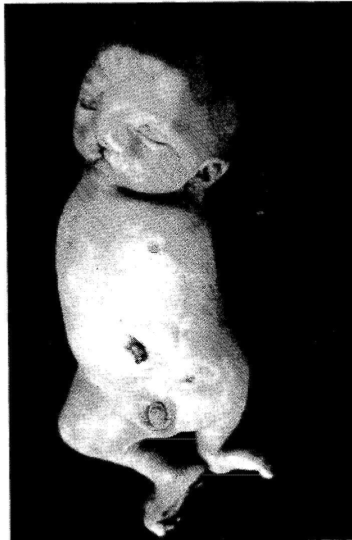


Figure 17-24. Diabetic embryopathy. Appearance of infant with amelia, "caudal regression" and cleft-lip. (From: Bruyere HJ Jr, Viseskul C, Opitz JM, Langer LO Jr, Ishikawa S, Gilbert EF: A fetus with upper limb amelia, "caudal regression" and Dandy–Walker defect. Europ. J. Pediat. 134:139–143, 1980.

Thalidomide embryopathy

Lenz [333] in Germany and McBride [334] in Australia first recognized the teratogenicity of thalidomide (alpha-phthalimidoglutarimide) in the human fetus. The embryo is most susceptible to the effects of thalidomide during the 23rd to 38th day after conception [335]. Similar malformations have been induced in rabbits [336] and in monkeys [337, 338]. Approximately 7000 children were affected by thalidomide [339]. The malformations include limb deformities in their most severe forms, i.e., phocomelia or amelia, congenital heart defects, ear abnormalities, intestinal atresias, anomalies of the eyes, and anomalies of the genitourinary tract [340]. The most common urinary tract anomalies include renal agenesis or hypoplasia, hydronephrosis, double kidney, and horseshoe kidney [341]. Cystic kidneys, renal ectopia and anomalies of rotation occur in others. Undescended testes and hypospadias in boys, and duplications of the uterine horns, hypoplasia of a uterine horn, and agenesis and double vagina in girls [345] are reported. McCredie [346–352] hypothesized the modus operandi of thalidomide embryopathy to be an interference with neural-crest-based sclerotomal organization; however, this hypothesis has been questioned.

CHROMOSOMAL DEFECTS

Renal lesions and associated abnormalities in chromosomal defects are listed on table 17-6.

Table 17-6. Chromosomal abnormalities*

Condition	Abnormalities of kidney and urinary tract
Autosomal	
Trisomy 21—Down syndrome (DS)	Renal dysplasia, nodular renal blastema, persistent fetal lobulation, retardation of maturation of the nephrogenic zone of cortex, hemangiomata, stricture of the ureteropelvic junction, hydronephrosis, focal cystic malformation of collecting tubules, immature glomeruli
Trisomy 18 sydrome	Cystic kidneys, horseshoe kidneys, ureteral duplication, renal duplication, renal dysplasia, renal agenesis, renal extopy, renal glomerulosclerosis and cystic tubules, persistent metanephric blastema, micromulticystic kidneys, retention of fetal lobulation, Wilms' tumor
Trisomy 13 syndrome	Duplication of kidneys and ureters, unilateral renal agenesis, stenosis of prostatic urethra, excessive renal arteries and veins, micromulticystic or pluricystic kidneys, excessive fetal lobulations, cystic dysplasia, segmental cystic dysplasia, cystic dilatation of collecting system, hydronephrosis, ureteropelvic junction atresia, Wilms' tumor
Trisomy 8 syndrome	Obstructive uropathy with hydronephrosis, posterior urethral valves with hydroureters and hydronephrosis, horseshoe kidney
Trisomy 9 syndrome	Bilateral cystic dysplastic kidneys, atresia of proximal ureters, rudimentary atretic urinary bladder, microcysts of kidneys, double ureters, bladder diverticulum
Triploidy	Micromulticystic kidneys, hypoplasia, hydronephrosis, cryptorchidism, hypospadias, labia majora like structures
Deletions	
5p (del(5p)) syndrome	Unilateral renal agenesis
18q (del(18q)) syndrome	Cryptorchidism and hypospadias in males, horseshoe kidneys, bilateral cortical nephroblastomatosis
9p (del(9p)) syndrome	Hydronephrosis and horseshoe kidneys, micropenis, hypospadias and/or cryptorchidism in males
11p (del(11p) syndrome	Wilms' tumor, sometimes bilateral, disorganization of renal parenchyma, medullary origin of Wilms' tumor
17p (del(17p)) (Miller–Dieker) syndrome	Bilateral double collecting system, hydronephrosis and abnormal calyceal patterns, fetal lobulations, cystic kidneys, renal agenesis
Duplications	
dup (10q)	Cystic renal dysplasia, hydronephrosis
dup (4p)	Unilateral hydronephrosis, pelvic displacement of kidneys with calyceal ectasia, bilateral intrarenal pelvis and excessive rotation of kidneys, hypoplastic kidneys
dup (20p)	Unilateral hydronephrosis with duplicated collecting system, hypospadias and cryptorchidism in males
Other rare chromosome abnormalities	
dup(3q), del(4p), del(11q), dup(3p), dup(10p), dup(12p), r(13), dup(13q), dup(14q), r(15)	Duplication of kidneys and/or ureters
dup(3q), dup(10q), (dup(1q), del(4p)	Cysts in kidneys

Table 17-6 (continued)

Condition	Abnormalities of kidney and urinary tract
dup(2q), dup(3q), dup(9p)	Horseshoe kidneys
dup(15q)	
dup(1p)	Ambiguous genitalia, hypoplastic kidneys
Sex chromosome abnormalities	
45,X (Ullrich–Turner) syndrome (UTS)	Horseshoe kidneys, double or clubbed renal pelvis, hypoplasia, hydronephrosis, bifid ureters, duplication of kidneys and/or ureters, unilateral renal agenesis, renal hypoplasia, retrocaval ureter with massive hydronephrosis, micromulticystic kidneys, membranoproliferative glomerulonephritis with persistent complement activation
47,XYY syndrome	Microcysts of kidneys, thin ureters, small bladder, cystic dysplastic kidneys
47,XXY, 48,XXXY, and 49,XXXXY (Klinefelter syndromes)	Cryptorchidism, small testes, and hypoplastic scrotum in males. No renal parenchymal abnormalities, hydronephrosis, hydroureter, and ureterocele
Chromosome abnormalities in renal-cell carcinoma	Abnormal calyceal collecting system, unilateral aplasia, cystic kidneys, renal dysplasia

*Associated malformations in these disorders include multiple congenital anomalies, minor anomalies, mild malformations, and frequently a typical phenotype.

Chromosomal abnormalities are associated with multiple congenital anomalies. Kidney and urinary tract abnormalities in patients with chromosomal aberrations are more common than in the general population.

Trisomy 21—Down syndrome (DS)

Developmental disorders of the kidney are uncommon in DS. Stricture at the ureteropelvic junction, hydronephrosis, and focal cystic malformation of the collecting tubules with immature glomeruli, [353] renal agenesis or hypoplasia, horseshoe kidney, hypoplastic kidney, and urethral valves [354] have been reported, as well as renal dysplasia and nodular renal blastema [355]. Small kidneys with glomeruli near the capsule characteristic of those of fetal and early postnatal life [356] have been described. Anatomical studies at the University of Wisconsin have confirmed the inferences of developmental delay in DS [357]. Retardation in maturation of the nephrogenic zone of the cortex and persistent fetal lobulation were the only consistent findings in our cases.

Trisomy 18 syndrome

Renal and urological abnormalities occur in 33% to 70% of cases in the trisomy 18 syndrome [358, 359]. Of 84 necropsies [360], 21% had horseshoe kidneys, 17% had urethral duplication, 11% had cystic kidneys, 9% had renal duplication, 7% had renal dysplasia or agenesis, and 3% had renal ectopy. Glomerulosclerosis and cystic tubules, often lined by primitive epithelium, and frequently seen; these findings are similar to those observed in children with congenital heart defects and infantile glomerulosclerosis [31]. Nests

of undifferentiated metanephric blastema resemble minute Wilms' tumor [361–363]. We have noted persistent fetal lobulation and occasional small glomerular and tubular cysts of the micromulticystic type described by Bernstein et al. [31]. Evidence of developmental delay is also present in other systems [364, 465]. It was suggested by Osathanondh and Potter [33] on the basis of microdissection studies that the cysts arise from multiple abnormalities of ductular and nephronic development. However, the evidence is not sufficiently compelling to exclude a secondary abnormality of formed tubules and glomeruli [4], since cysts are barely visible at birth and become larger with age [366]. The cysts are present throughout the nephron and are associated with proliferative and regressive changes [367].

A Wilms' tumor was reported in a 13-year-old girl with trisomy 18 [368].

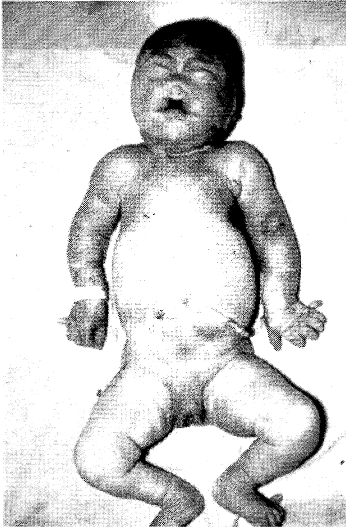
Trisomy 13 syndrome

Renal abnormalities have been described in 50% to 60% of individuals with trisomy 13. Cystic kidneys were observed by Warkany et al. [360]. Osathanondh and Potter [33] described cystic kidneys of the adult polycystic type (their type 3) in one case. Duplication of kidneys or ureters, unilateral renal agenesis, and stenosis of the prostatic urethra have been also reported [369]. In the original descriptional of the trisomy 13 syndrome, Patau et al. [370] observed a persistent urachus.

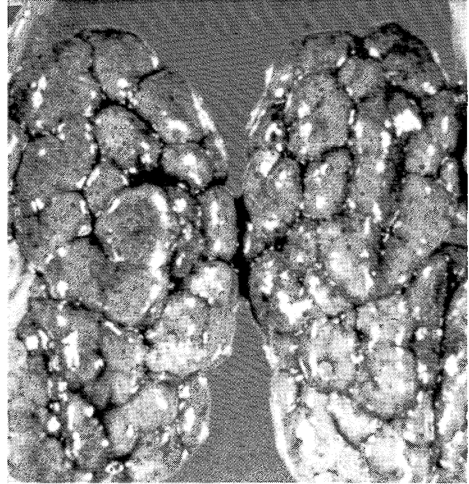
Accessory renal arteries and veins have been noted [366]. The most consistent findings are excessive fetal lobulations and glomerular and tubular cystic lesions (figure 17-25), which may be micromulticystic or pluricystic and which are usually confined to the cortex but occasionally are present in the medulla. Although cystic dysplasia of the kidneys is infrequent and usually segmental in trisomy 13, we have seen a case of cystic dysplasia of such severe degree as to be incompatible with life. Bartman and Barraclough [371] found cysts originating from glomeruli and Barraclough [371] found cysts originating from glomeruli and from the collecting system in which isolated cystic dilatations with no relationship to the rest of the nephron were present. In the collecting system, the tubular cysts represented ampullary dilatations; occasional giant nephrons were present, but the convoluted tubules and loops of Henle were unaffected. In the medulla, cystic tubules surrounded by embryonic mesenchyme were reminiscent of structures in cystic dysplastic kidneys. Hydronephrosis has been noted [369, 372]. We observed an infant with trisomy 13 with severe skeletal anomalies and cystic dysplastic kidneys whose parents were taking LSD at the time of conception and during pregnancy.

Trisomy 8 syndrome (Warkany syndrome)

The chromosomal mechanism accounting for the Warkany syndrome (WS) includes either complete trisomy 8, usually with mosaicism of normal cells, or translocation leading to partial trisomy 8 [373–376]. Clinical characteristics include mental retardation, distinctive facial appearance, absent or dysplastic



A



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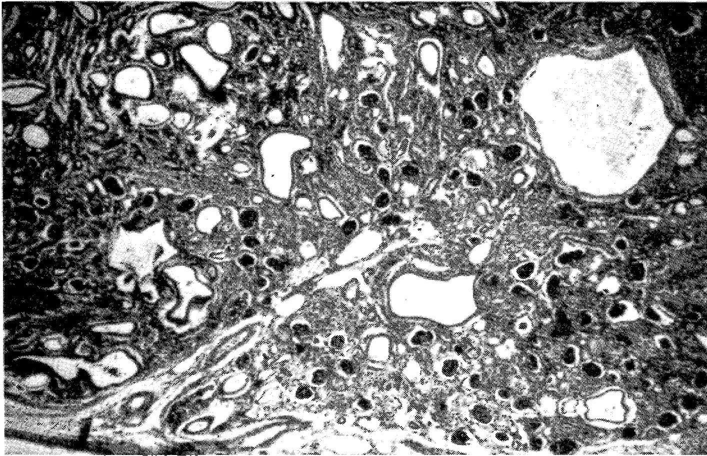

C

Figure 17-25. Trisomy 13 syndrome. **A.** Clinical appearance of an infant with bilateral cleft-lip and absent premaxilla, characteristic face, and polydactyly. **B.** Gross appearance of the kidney in trisomy 13 showing excessive fetal lobulations and multiple small subcapsular cysts. **C.** Microscopic appearance of the kidney in trisomy 13 showing small glomerular and tubular cysts—micromulticystic kidney. H&E Mag X100. (A and B from: Gilbert, EF, Opitz JM: Chapter 64, Renal Involvement in Genetic–Hereditary Malformation Syndromes. In: Hamburger J, Crosnier J, Grunfeld JP (eds): Nephrology. New York: John Wiley & Sons, 1979, pp. 909–944.

patellae, joint contractures, deep plantar/palmar skin furrows, vertebral defects, narrow pelvis, and distinct toe deformities. Severe ureteral and renal anomalies occur, predominantly obstructive uropathy with vesicourethral reflux and hydronephrosis and secondary chronic pyelonephritis, horseshoe kidney, and fixed pelvis [377, 378]. We observed an infant who died shortly after birth with complete trisomy 8 and who had posterior urethral valves producing partial obstruction, hydronephrosis and hydroureters, and dilatation of the bladder.

Trisomy 9 syndrome

The first living infant with probable full trisomy 9 was reported by Juberg et al. [379]; the infant had microcephaly, flat nasal bridge, epicanthal folds, micrognathia, low-set ears, and cleft palate. Bilateral cystic dysplastic kidneys with atresia of the proximal ureters and a rudimentary atretic urinary bladder were observed. A case reported by Blair [380] had, in addition, hepatic and pancreatic dysplasia, cystic dysplastic kidneys, and atretic ureters. These cases were recorded before Giemsa banding studies were available, but the phenotype of these cases is consistent with 9 trisomy [381–384].

Trisomy 9 mosaicism syndrome [385]

Trisomy 9 mosaicism is associated with joint contractures, congenital cardiac defects, low-set, malformed ears, sloping forehead, deeply set eyes, and micrognathia. Genitourinary abnormalities include micropenis, cryptorchidism, bladder diverticulum, double ureters, and microscopic renal cysts lined with epithelium [386].

Triploidy

Triploid individuals have three haploid sets of chromosomes (i.e., a total of 69). About two thirds of the cases have been XXY and about one third XXX, rarely XYY [387]. Triploidy is estimated to occur in about 1% of conceptuses [388]. Most are spontaneously aborted [387, 389]; a few have been liveborn. Triploidy is not maternal-age-dependent. Commonly triploid pregnancies are associated with imminent abortion, polyhydramnios, proteinuria, hypertension, edema, and midtrimester preeclampsia [390]. One 69, XXX infant lived for 26 days [391]. Diploid/triploid mosaic infants may survive with moderate to severe mental retardation [392, 393]. A triploid infant is characterized by a large posterior fontanel as a result of underdevelopment of occipital and parietal bones. Enlarged head circumference, hydrocephalus, and CNS malformations including agenesis or hypoplasia of the corpus callosum, holoprosencephaly [394], cerebellar anomalies, Arnold–Chiari malformations, and meningomyelocele [395, 396] are frequent. Cleft lip or cleft palate is found in one third of cases. Hypoplastic and hydronephrotic kidneys, cystic dysplasia, cryptorchidism, and Leydig cell hyperplasia have been described [397]. Per-

ineal hypospadias, scrotum bifida, and labia majora like structures have been seen in 69,XXY triploid infants who were sex-chromatin negative, i.e., in those in whom inactivation of an X-chromosome occurred.

A partial hydatidiform molar placenta is present in 85% of triploidies; all partial moles may be considered triploidies until proven otherwise [398–400].

We have observed a liveborn triploidy infant with microcystic renal disease involving glomeruli and tubules [40].

Chromosome 4 short arm deletion (4p- or Wolf–Hirshhorn syndrome)

Renal abnormalities occur in about one third of affected patients [359] and consist of renal agenesis or hypoplasia, nonfunctioning kidney, dilated collecting system, vesicoureteral reflux, and chronic pyelonephritis [359, 401, 402]. Hypospadias are found constantly in this syndrome [359, 403, 404].

5p- or del(5p) syndrome

This syndrome, described by Lejeune et al. [405], has an incidence of 1/50,000 births. It is characterized by a cat-like cry in infancy, microcephaly, characteristic facial appearance with downward slant of the palpebral fissures, a low birth weight, mental deficiency, microcephaly, round face, hypertelorism, and frequent congenital heart defects. Renal anomalies occur in about 40% of individuals. Unilateral renal agenesis has been described [405–407] as well as ectasia of distal tubules, boneslice kidney, and duplication of the urinary tract.

18q- or del(18q) syndrome

The del(18q) syndrome is characterized by mental retardation, narrow ear canals, hypotonia, short stature, peculiar facial appearance, and an increased number of whorls in the fingerprint pattern. Cryptorchidism and hypospadias may be present in the male; horseshoe kidney has been described [408].

We observed bilateral cortical nephroblastomatosis in this syndrome. This has been reported also in partial dup(20p) [409, 410].

9p- or del(9p) syndrome

This syndrome is characterized by craniostenosis involving the metopic suture leading to trigoncephaly; up-slanting palpebral fissures; prominent eyes secondary to hypoplastic supraorbital ridges; midface hypoplasia with a short nose, depressed nasal bridge, and anteverted nares; and frequent cardiovascular defects [411–413]. Most cases have had severe mental deficiency. Micropenis, hypospadias, and cryptorchidism may be present in males and in some cases, hydronephrosis and horseshoe kidneys [414–416].

11p- or del(11p) syndrome and Wilms' tumor

Interstitial deletion of the distal half of band 11p13 leads to aniridia and predisposition to Wilms' tumor in a characteristic deletion syndrome [417–419]

of mental retardation. Multiple minor anomalies, congenital heart defect, cryptorchidism in males, and predisposition to other tumors occur.

There is genetic heterogeneity of aniridia and of Wilms' tumor, which is a neoplastic dysplasia that may result from a variety of embryologic insults, some of which may be chromosomal or heritable [420–422].

The presence of a del(11)(p13) confirms the diagnosis of the Wilms' tumor/ aniridia syndrome and indicates a high risk of Wilms' tumor [422]. The Wilms' tumor appears histologically similar to that in typical Wilms' tumor patients. The evaluation, follow-up, and ultimate prognosis of del(11p) patients remain to be established. We observed disorganization of the renal parenchyma with the tumor occupying the medulla of the kidney in contradistinction to the usual cortical origin of Wilms' tumor. In our patient, Wilms' tumor development seemed to occur in response to human chorionic gonadotropin (hCG) treatment for cryptorchidism; indeed, in vitro the tumor was found to be dependent on hCG for growth and cell division. Hence, caution is recommened in using hCG to treat cryptorchidism in this syndrome.

Wilms' tumor and iris dysplasia, with apparently normal chromosomes [423] and no gene loss demonstrable by enzyme markers and DNA analysis, have been reported [424]. Aniridia seems to define a risk for Wilms' tumor even in the apparent absence of del(11)(p13). It has not yet been determined whether patients with Wilms' tumor and aniridia exhibit subcapsular renal dysplasia as found in children with the hereditary form of Wilms' tumor [425].

17p- or del(17p) (Miller–Dieker syndrome)

The Miller–Dieker syndrome (M–DS, lissencephaly Type I) is characterized by smoothness of the surface of the brain, microcephaly, slight internal hydrocephalus, a severe disturbance of development of gray matter with four instead of six cortical layers, minor facial anomalies with characteristic facial appearance, occasional hirsutism, clouding of cornea, polydactyly, and variable malformations of other organs [426–436].

Dobyns [437, 438] delineated at least two and probably more pathological types of lissencephaly. Type I (classical lissencephaly) includes three subtypes: 1) the M–DS, 2) Norman– Roberts syndrome, and 3) isolated lissencephaly sequence. Type II lissencephaly includes those cases with agyria, obstructive hydrocephalus with additional severe brain malformations, including Walker–Warburg (HARD ± E) syndrome and the Neu–Laxova syndrome.

The chromosome abnormality is restricted to chromosome 17 (rings or deletions) or unbalanced translocations resulting in partial deletion of 17p in addition to duplication of some other autosomal segment. Two patients reported by Dobyns [439] had normal chromosomes. He suspected that there may be submicroscopic deletions of 17p in these cases.

Congenital heart disease, agenesis of one kidney [432], fetal lobulation, and cystic kidneys [427, 431] have been described in the M–DS but not in the other subtypes of type I lissencephaly.

In two of four cases reported by Van Allen and Clarren [439], renal abnormalities included bilateral double collecting systems, hydronephrosis, and abnormal calyceal patterns.

22q- (del(22q)) (cat-eye syndrome)

Cat-eye syndrome is characterized by coloboma of the iris, anal atresia, preauricular tags, and an extra small marker chromosome that is thought to be derived from chromosome 22 [440]. Renal abnormalities occur in 60% to 100% of these patients and consist of renal agenesis, hypoplasia and cystic dysplasia, and horseshoe and pelvic kidney; ureteropelvic junction obstruction, vesico-ureteral and urethral stenosis, ectopic ureterovesical orifice, bladder neck obstruction with reflux, abnormal shape of renal pelvis, hypoplastic urinary bladder, and chronic pyelonephritis [359, 440–442].

Duplications

dup(10q) syndrome

Yunis and Sanchez [443] described a six-year-old white boy with severe growth and mental retardation, hypotonia, short neck, cervical and thoracolumbar scoliosis, bow-shaped and thin upper six ribs, mild osteoporosis, ventricular septal defect of the atrioventricular canal variety with moderate pulmonary hypertension, bilateral inguinal herniae, probable hypoplasia of the right kidney, hypoplasia of right collecting system and proximal ureter, bilateral inguinal testes, microphthalmia, bilateral lens opacity, and blindness due to complete replacement of the retina by fibrous tissue. The face was flat and round with wide-set eyebrows, apparently low-set ears, small nose with depressed nasal bridge, highly arched palate, and micrognathia. The patient had a duplication of bands 10q24–26. The mother's first pregnancy resulted in a spontaneous abortion, and one other therapeutically aborted female infant had a similar phenotype. Therefore, mother or father must have been a translocation carrier. Three other similar cases have been reported [444, 445].

Juberg et al. [446] reported a twin pregnancy that resulted in the delivery of a phenotypically normal female and an abnormal male with dup(10q)del(12p), microphthalmia, microcephaly with intracranial malformations, a complex cardiac anomaly, imperforate anus, and bilateral hydrocele. Renal anomalies included cystic renal dysplasia with bilateral hydronephrosis.

dup(4p) syndrome

The most consistent findings include obesity, mental and motor retardation, seizures, microcephaly, bulbous nose with depressed or flat nasal bridge, synophrys, macroglossia, irregular teeth, small pointed mandible, enlarged ears with abnormal helix and antihelix, and short neck [447]. Micropenis, hypospadias, and cryptorchidism may occur in males.

Unilateral hydronephrosis, pelvic displacement of the kidneys with calyceal ectasia, and excessive rotation of both kidneys have been noted [448].

Hypoplastic kidneys and hydronephrosis [449] and cortical microcysts [450] have been described.

dup(20p) syndrome

dup(20p) syndrome is characterized by blepharophimosis, large and abnormally formed ears, cubitus valgus, frequent vertebral defects, and genital hypoplasia with cryptorchidism [417, 451]. Urogenital malformations include unilateral hydronephrosis with duplicated collecting system and hypospadias [452].

Other rare chromosome abnormalities

Hydronephrosis due to ureteral obstruction is frequent in dup(3q), dup(4q), dup(5p), dup(8q), del(10p), r(10), del(11q), dup(17p), and dup(19q) [451].

Duplication of kidneys and/or ureters is frequent in dup(3q), del(4p), and del(11q), and occasionally may be present in dup(3p), dup(10p), dup(12p), r(13), dup(13q), dup(14q), and r(15) [451].

Cysts in the kidneys may be present in dup(3q), dup(10p), dup(1q), and del(4p) [451].

Horseshoe kidneys have also been described in dup(2p), dup(3q), dup(9p), and dup(15q) [451].

We observed a case of a tandem duplication within the short arm of chromosome 1 in a child with ambiguous genitalia and multiple congenital anomalies including hypoplastic kidneys [453].

SEX CHROMOSOME ABNORMALITIES

45,X gonadal dysgenesis (Turner syndrome)

The Turner phenotype includes short stature, broad chest with wide spacing of nipples, ovarian dysgenesis with hypoplasia or absence of germinal elements, congenital lymphedema with residual puffiness of the dorsum of the fingers and toes (80%), anomalous ears, webbed posterior neck (50%), cubitus valgus, excessive number of pigmented nevi (50%), and cardiac defects (20%). Renal anomalies occur in 76% of cases, most commonly horseshoe kidney, double or clubbed renal pelvis, hypoplasia or hydronephrosis and bifida ureters, duplication of kidneys and ureters, unilateral renal agenesis with abnormalities of the contralateral kidney, and renal hypoplasia [40, 85, 359, 454–459]. A retrocaval ureter with massive hydronephrosis was reported [460]. Goodyer et al. [461] reported persistent complement activation and membranoproliferative glomerulonephritis in the syndrome with 46X,del(X)(p11). Micromulticystic renal disease has also been observed [40].

47,XYY syndrome

Prevalence among male newborns is about 1:1000 [451, 462, 463]. Tallness, borderline intelligence, and aggressive behavior are present in some cases. The kidneys are described as symmetrically enlarged, with small cysts 0.1 to 0.8 cm in diameter throughout the parenchyma [463], lined with undifferentiated

epithelium. Some contain several glomerular tufts. Connective tissue found between the cysts had abnormal glomeruli, the ureters were very thin but not atretic, and the bladder was small, cylindrical, and empty, consistent with cystic dysplastic kidneys.

47,XXY, 48,XXXY and 49,XXXXY (Klinefelter syndromes)

The incidence of Klinefelter syndrome is one in 500 newborn males. The phenotype is characterized by tallness (in the higher aneuploidies there may be shortness of stature), hypogonadism and hypogenitalism; the greater the degree of aneuploidy, the more severe are its effects [461]. These patients have been reported to have genitourinary abnormalities including small penis (80%), small testes (94%), hypoplastic tubules, diminished Leydig cells, cryptorchidism, and hypoplastic scrotum (80%) [464]. Kidney cysts, hydronephrosis, hydroureters, and ureterocele have been noted on rare occasions [359].

Chromosome abnormalities in renal-cell carcinoma

Familial predisposition to renal-cell carcinoma has been well documented. In a cytogenetic study of a family prone to renal-cell carcinoma, Cohen et al. [465] described five affected members with a balanced reciprocal translocation between chromosomes 3 and 8 in peripheral leukocytes.

Cytogenetic studies performed on the direct chromosome preparations of renal-cell carcinoma cells and cultured peripheral blood lymphocytes of a patient with familial renal-cell carcinoma showed a specific, acquired translocation (3p;11p) present in the majority of metaphases.

Thus, renal-cell carcinoma is another example of a chromosome deletion occurring germinally or somatically in association with a specific tumor (others being retinoblastoma and Wilms' tumor). This adds further support to the presence and action of specific human oncogenes [305].

Kantor et al. [466] found a pericentric inversion of chromosome 2 (p13;q11) in a man with unilateral renal carcinoma at age 42 years; his mother had cancer of the colon. A constitutional abnormality of chromosome 9, resulting from a probable inversion with subsequent duplication of p11;q13, was found in a man with unilateral renal carcinoma at age 43; his mother had breast cancer. A woman with bilateral renal carcinoma diagnosed at age 60 years had chromosomal mosaicism for the Turner syndrome (46,XX/45,X/47,XXX). An intravenous pyelogram showed congenital abnormalities of the renal calyceal collecting system. Familial renal carcinoma may also occur in the absence of chromosome abnormalities, as recently reported in 10 persons in one family [467].

In a familial 11/13 translocation [468], the cause of death was attributed to biliary atresia in an infant with unknown chromosome constitution and multiple congenital anomalies. The father was a balanced heterozygote. In another relative, aplasia of the left kidney was associated with myelomen-

ingocele and congenital heart disease. Cystic kidneys and renal dysplasia were not reported.

CONCLUSIONS

Renal anomalies are common, though variable, in many syndromes. Since many of these have genetic or teratogenic implications, careful documentation is necessary.

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18. THE MOLECULAR BIOLOGY OF COMPLEMENT DEFICIENCY SYNDROMES

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The complement system constitutes an important effector of host defenses but may also serve as a mediator of immunopathological events. Genetically determined human complement deficiencies were first recognized about 25 years ago. These observations, coupled with advances in protein chemistry and molecular biological studies, have stimulated great interest in the genetic disorders of the complement system. As a consequence, better understanding of the biological function and role of complement in human disease has been facilitated.

The complement system is a set of proteins (table 18-1) that, in conjunction with specific antibodies, constitutes a primary humoral defense system against bacterial and viral infections. Activation of the complement cascade results in the expression of at least four distinct biological activities: 1) enhanced phagocytosis of complement-coated target cells, 2) irreversible structural and functional damage to target-cell membranes, 3) activation of specialized physiological and cellular functions, and 4) solubilization and metabolism of immune complexes. The complement cascade is activated, and the biological properties of complement are generated by two distinct pathways, the classical and alternative pathways, which converge to form a shared pathway sequence (figure 18-1). For details, the reader is referred to several recent reviews [1–7].

The classical pathway is activiated by immune complexes containing IgG or IgM antibodies but also by viruses [8–11], DNA, C-reactive protein [12, 13], and heart mitochondrial membranes [14, 15]. The alternative pathway is

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Table 18-1. Proteins, regulators, and receptors of the complement system

Complement protein	Molecular[a] mass (daltons)	Subunit structure	Serum[a] concentration (ug/ml)	mRNA[a,b] size (Kb)	Gene[a] size (Kb)
C1q	410,000	6A; @24,000 6B; @23,000 6C; @22,000	70	A; N.D. B; 1.5 C; N.D	A; N.D. B; 2.6 C; N.D.
C1r	95,000	Single chain	35	2.0	N.D.
C1s	87,000	Single chain	35	2.0	N.D.
C2	110,000	Single chain	25	2.9	18
B	93,000	Single chain	200	2.6	6
D	24,000	Single chain	1	1.0.	N.D.
C3	185,000	α; 110,000 β; 75,000	1500	5.2	24
C4	200,000	α; 93,000 β; 78,000 γ; 33,000	400–600	5.3	A; 22 B; 16/22
C5	190,000	α; 115,000 β; 75,000	75	5.5	> 70
C6	115,000	Single chain	75	N.D.	N.D.
C7	115,000	Single chain	65	3.9	N.D.
C8	163,000	α; 64,000 β; 64,000 γ; 22,000	55	α; 2.5 β; 2.6 γ; 1.0	α; N.D. β; N.D. γ; N.D.
C9	71,000	Single chain	60	2.4	80
C1–INH	104,000 (34% CHO)	Single chain	150	1.8	17
P	~224,000	6; 56,000	25	1.6	N.D.
C4–bp	500,000	7; 70,000 (6α; 70,000) (1β; 45,000)	~150	2.5	20
I	88,000	46,000 39,000	35	2.4	N.D.
H	155,000	Single chain	500	4.4	>100★
S-protein	~80,000	Single chain	500	1.6	N.D.
Membrane receptors and regulators					
DAF	70,000	Single chain	—	3.1 2.7 2.0	N.D.
MCP	58,000–63,000	Single chain	—	4.2	N.D.
HRF	65	Single chain	—		
CR1	A; 190,000[c] B; 220,000 C; 160,000 D; 250,000	Single chain	—	A; 8.6 B; 11.6 C; 7.3 D; 12.8	N.D.
CR2	140,000	Single chain	—	5.0	N.D.
CR3	260,000	α; 165,000 β; 95,000	—	α; 6.0 β; 3.0	α; N.D. β; 32
C5aR	~45,000	N.D.	—	N.D.	N.D.
C1qR	~70,000	N.D.	—	N.D.	N.D.

[a] Approximate values.
[b] Only major mRNAs are shown.
[c] Allotypes.
★ Murine gene (human unknown)

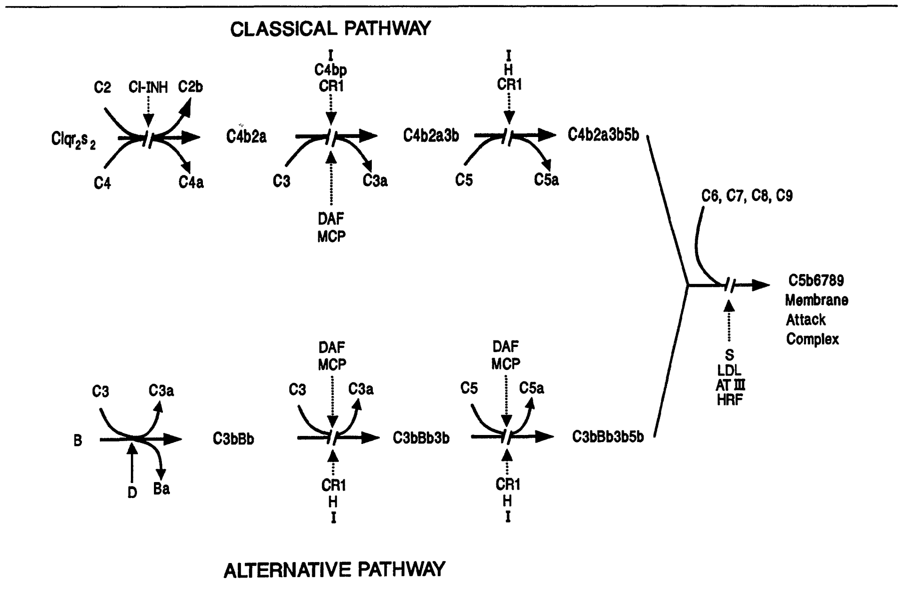

Figure 18-1. Pathways of complement activation. Sites of regulation are depicted by dashed arrows. The regulator proteins are shown above or below the dashed arrows.

activated by such bacterial and yeast polysaccharides as endotoxin [16] or zymosan [17] and by some viruses. The activation of either pathway results in the formation of an enzyme, C3 convertase, that cleaves C3 into C3a, a vasoactive peptide, and C3b. Interaction of cell-bound C3b with regulator proteins H and I and the receptor CR3 enhances phagocytosis by CR3-bearing cells. In addition, C3b is a component of the C5 convertase enzyme complex that helps to solubilize immune complexes, and participates in immune-complex clearance by C3b receptor (CR1) bearing cells. Activation of either pathway also results in the cleavage of C5 into two fragments, C5a, a chemotactic peptide that induces neutrophil degranulation, and C5b, which mediates the assembly of the lytic C5-9 complex. The existence of these two activation pathways explains why a deficiency in one of the early components in either of the pathways does not prevent the activation of C3 and generation of the lytic complex, C5-9. Also, if one of the proteins of the C5-9 complex is deficient, either pathway can be activated through C3, but assembly of the membrane attack complex is blocked. The absence of a regulatory protein (for example, H or I for the alternative pathway, or C1 inhibitor (C1-INH) for the classical pathway) results in sustained spontaneous activation of the corresponding pathway.

Several of these complement-dependent functions are particularly relevent to the pathogenesis of renal immunopathology. Chief among these is the role

of complement in the disposition of immune complexes (reviewed in [18]). Activation of the complement system prevents precipitation [19] of antigen antibody complexes and promotes solubilization of preformed antigen antibody precipitates [20]. Although most of the studies supporting this hypothesis have been performed in vitro, the importance of C3 in clearance of complexes from the kidney was established [21] in an experimental serum sickness model. The complement system also serves as a buffer limiting the absolute concentration of soluble complexes free in plasma by virtue of interactions of C3b receptors (CR1) on erythrocytes and C3b protein covalently bound to the immune complex. This interaction is reversible so that removal of the complex from the erythrocyte by the reticuloendothelial system, primarily in the liver, regenerates erythrocyte acceptors for additional binding of immune complex.

Complement also has an important function in regulating leukocyte adhesion, activation, and noncytotoxic release of leukocyte products. These effects and the modifying action of complement on immune responsiveness [22, 23] constitute the major relationships between complement and the kidney. However, the function of the complement system in defense against bacteria and viral pathogens or direct damage to host cell membranes may also be relevent in understanding the genetics of complement and renal pathology.

COMPLEMENT DEFICIENCIES: GENERAL CONSIDERATIONS

Deficiencies of almost all the complement components have been reported (table 18-2). With two exceptions, the complement deficiencies are inherited as autosomal recessive traits (codominant when analyzed at the protein level). The exceptions are deficiency of C1-INH (hereditary angioneurotic edema), which is inherited as an autosomal dominant trait, and properdin deficiency, inherited as an X-linked disorder. All but one of the complement deficiencies are associated with clinical symptoms; C9-deficient individuals are asymptomatic.

Deficiencies of the early complement components C1q, C1r, C1s, C2, and C4 are generally associated with rheumatic disorders [24–26]. These include

Table 18-2. Clinical conditions associated with complement deficiencies

Clinical manifestations	Associated complement deficiency
Glomerulonephritis	C1q, C3
SLE or SLE-like symptoms	C1q, C1r, C1s, C4, C2
Angioneurotic edema	C1–INH
Increased susceptibility to infection	
i) Pyogenic encapsulated bacteria	C3, I, P, CR3
ii) Neisseria	C5, C6, C7, C8
Paroxysmal nocturnal hemoglobinuria (PNH)	DAF

discoid lupus, systemic lupus erythematosus, glomerulonephritis, dermatomyositis, anaphylactoid purpura, and nonspecific vasculitis. The pathophysiologic basis for the association between these rheumatic disorders and complement deficiencies is unclear, but a number of hypotheses have been proposed: 1) a theoretical impairment of host defenses against viral agents; 2) the class III major histocompatibility genes (C2, C4) are in linkage disequilibrium with specific HLA haplotypes [27–31], and thus the deficiency may simply be genetically linked to a gene(s) primarily involved in the autoimmune disorder; 3) as indicated above, the role of complement in processing immune complexes [18] may be impaired; and 4) the function of complement in immune response may be altered.

There are several important differences between rheumatic diseases seen in complement-deficient patients and noncomplement-deficient individuals. For example, the SLE-like illness found in association with complement deficiency is characterized by an early onset (often in childhood), of prominent annular photosensitive skin lesions resembling discoid lupus, relatively limited renal and pleuropericardial involvement, and an infrequent deposition of immunoglobulin and C3 in the skin [24–26]. In addition, sera from complement-deficient individuals with SLE contain low levels of antinuclear antibodies and antinative DNA antibodies [26, 32–34]. In contrast, anti-Ro (SSA) antibodies are found more frequently in the complement deficient patients [33, 34].

Deficiencies of C3, factor H, and factor I predispose to recurrent infection with encapsulated pyogenic bacteria. Individuals lacking the terminal components C5, C6, C7, and C8 display increased susceptibility to recurrent or disseminated Neisserial infections. C1-INH deficiency results in angioneurotic edema.

With the advent of molecular biology, it has become possible to examine mechanisms accounting for these deficiencies at the genomic level and to address more comprehensively the reasons for association of disease with the deficiency. Except for properdin and C6, cDNA clones have now been obtained for all complement components, including the complement receptors and regulatory proteins. The following sections of this review will discuss each complement deficiency with emphasis on present knowledge of the molecular genetic defect.

COMPLEMENT GENE FAMILIES

A convenient framework for understanding complement is to group the complement components in supergene families based on smilarities in structure, function, and chromosomal localization (figure 18-2; reviewed in [35]). For instance, the genes encoding C2, factor B, and C4 constitute the class III genes of the major histocompatibility complex (MHC) on human chromosome 6 (figure 18-3). The 3′ terminus of the C2 gene is upstream of, and close to, the 5′ terminus of the factor B gene (~400 base pairs) [36]. The two C4 genes (C4A and C4B) lie approximately 30 Kb downstream from the C2 and

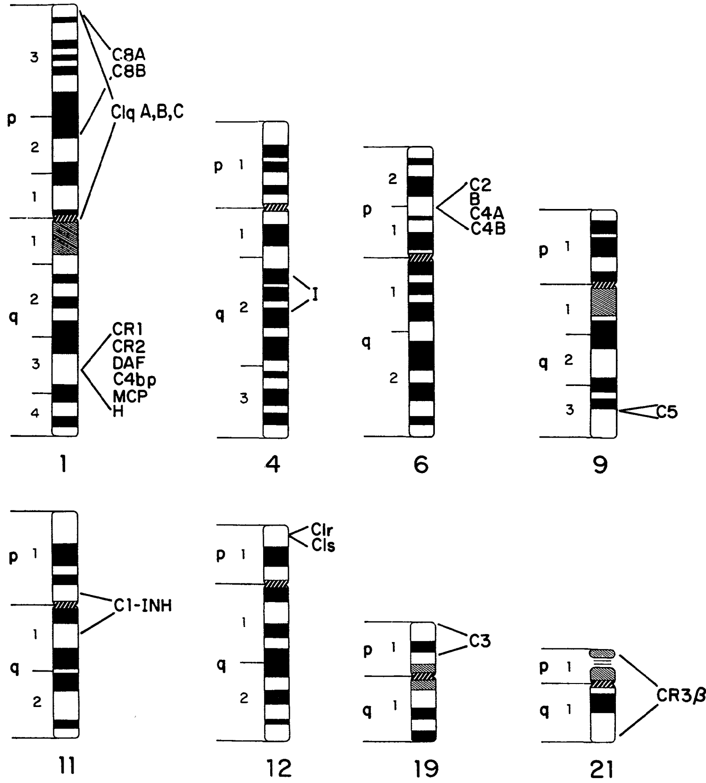

Figure 18-2. Chromosomal location of the human complement genes.

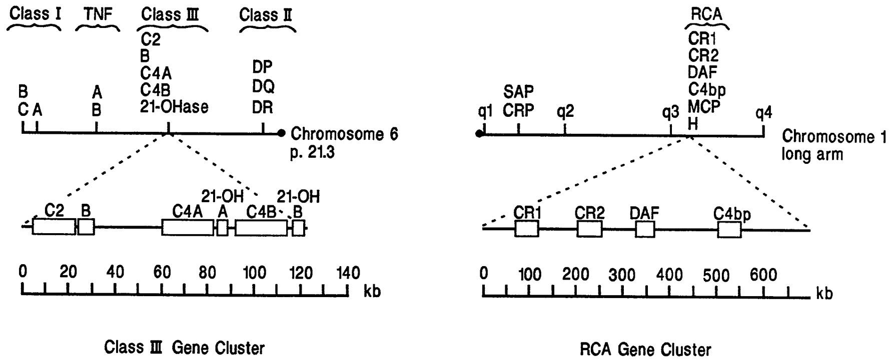

Figure 18-3. Map of the MHC Class III and RCA genes. The centromeres are indicated by dark circles. TNF: tumor necrosis factor; 21-OH: 21-hydroxylase; CRP: C-reactive protein; SAP: serum amyloid P.

factor B genes. The C4 loci are separated by 10 Kb, and each has a cytochrome P-450 steroid 21-hydroxylase (21-OH) gene within 1.5 Kb of the 3′ terminus [37–39]. The order of the genes in the direction of transcription is C2-BF-C4A-210HA-C4B-210HB, and they have been mapped within a 0.7 centimorgan region between HLA-B and HLA-DR [40, 41]. Factor B and C2 are similar in structural and functional features, suggesting that the genes were derived from a common ancestral gene [42]. The topology of the MHC class III genes is highly conserved in evolution, as shown in several studies of the corresponding region in mice [43, 44].

The genes encoding complement receptors CR1 and CR2, C4-binding protein, factor H, and decay-accelerating factor constitute members of another supergene family clustered on the long arm of chromosome 1 [45–48] (figure 18-2). Moreover, their products share several structural and functional characteristics. Sequence analyses of the coding regions of these genes demonstrate repeating homology units of approximately 60 residues, which are also seen in the sequence of C2 [42], factor B [49, 50], and C1r [51]. These repeating homology units are also found in non-complement proteins such as the IL-2 receptor, beta-2-glycoprotein 1, the haptoglobin alpha chain, and the herpes simplex virus glycoprotein C. The presence of these repetitive homologous sequences in herpes simplex virus glycoprotein C may reflect fortuitous or directed insertion of the viral genome at one or several of the loci of this gene family during evolution—i.e., homologies not based on divergence from an ancestral gene but based on encounter with infectious agents.

The genes for complement proteins C3, C4, and C5 share similar gene structure and primary protein structural and functional properties with alpha-2-macroglobulin and the pregnancy zone protein. These genes all encode multichain disulfide-linked molecules. Except for C5, a thiolester reactive site is a distinct characteristic of this family.

Based on primary sequence, several of the proteins of the membrane attack complex also appear to be members of a single gene family [52–55]. That is, the C8 alpha chain, the C8 beta chain (encoded by separate messenger RNAs), C9, and the cytolytic protein, perfoin, that is derived from natural killer cells display sequence and organizational homology.

C1 inhibitor shares structural and functional characteristics with several serine proteinase inhibitors, so it is often included in the SERPIN (serine proteinase inhibitor) supergene family (see below).

PROTEIN POLYMORPHISMS

Approximately two decades ago, genetically determined electrophoretic variants of the C3 protein were observed [27]. The common C3 variants are found in all major racial groups, and about 20 rare variants have been detected. Other methods have been used to detect, at the protein level, genetic variants of the other polymorphic complement proteins such as factor B, C2, C4, C6, C7, C8, and factor D [27]. In some instances the structural basis for this

genetic variation has been determined by sequence analysis of cDNA clones [56]. Associations between polymorphic forms of individual components of complement and certain diseases, including several renal disorders, have been recognized (reviewed in [27, 28, 57]), but the basis for the associations is not known.

Considerable attention has been given to an analysis of the complement proteins encoded by genes within the MHC. Studies of protein polymorphisms of the C2, factor B, C4A, and C4B loci, together with typing of the class I and class II genes, have generated the concept of *extended* haplotypes [29]. Extended haplotypes are defined as chromosomes bearing decreased frequency of recombinant events extending over approximately a 10-million base-pair region of chromosome 6. Taken together, the extended haplotypes account for nearly 30% of haplotypes in normal Caucasian populations. These observations have relevance for the well-recognized association of a number of diseases with certain extended haplotypes and the remarkable finding that mixed lymphocyte cultures from unrelated individuals sharing the same extended haplotypes exhibit low or absent reactivity [58]. The latter has obvious implications for transplantation biology [30, 31].

DEFICICENCY OF THE FIRST COMPONENT (C1)

The C1 subunits, C1q, C1r, and C1s, form a calcium-dependent macromolecular complex composed of one C1q molecule and a complex of two C1r and two C1s molecules [59]. C1r and C1s, the zymogen forms of serine proteases, are each 83,000-dalton single-chain polypeptides. cDNAs encoding C1r [60] and C1s [61, 62] have been cloned and the primary structure of the proteins deduced from sequence analysis. Internal repeating structural domains found in both C1r and C1s are similar in structure to motifs observed in the complement regulatory proteins and C2 and factor B. The C1r and C1s genes are closely linked on chromsome 12p [61, 63]. The C1q molecule is a 410,000-molecular-weight molecule composed of three qualitatively distinct chains A, B, and C, of molecular mass 24,000, 23,000, and 22,000, respectively. The fully functional C1q molecule is composed of six molecules each of the A, B, and C chains. cDNAs for the A [64] and B [65] chains have been cloned; hence, the C1q polypeptide chains are each coded by separate genes. Each of these genes has been localized on chromsome 1p. The C1q B gene is 2.6 Kb long and has a single 1.1-Kb intron [65].

Deficiency of C1 is a complex phenomenon at the molecular level, since five separate genes encode the C1 macromolecule. Each of these genes is under separate control and regulation so that abnormalities in one may result in complete or partial C1 functional deficiency. A limited number of individuals with C1q deficiency have been reported [24, 66]. However, it is clear that C1q deficiency is inherited as an autosomal codominant trait that generally leads to markedly reduced levels of serum total hemolytic complement and C1 functional activity. At least two distinct forms of C1q deficiency have been

recognized [66]. In one, no C1q can be detected. In the other, a nonfunctional C1q protein is present. Studies of the dysfunctional C1q in two different families show that it is antigentically deficient when compared to normal C1q [67, 68]. The dysfunctional C1q does not bind to immunglobulin [67, 69], nor does it interact with C1r and C1s. The molecular masses of C1q in two affected kindreds differ from each other as well as from normal C1q [67, 69]. A lupuslike clinical syndrome and increased susceptibility to infection had been observed in C1q-deficient individuals.

An autosomal codominantly inherited deficiency of C1r has been described in which C1r is markedly reduced (<1% of normal) and C1s is moderately reduced (between 20% and 50% of normal) [24, 66]. This leads to reduced C1 functional activity with normal C1q levels. The basis for the association of the moderately reduced levels of C1s with an absence of C1r is unknown. The clinical features of C1r/C1s deficiency are similar to those found in the other deficiencies of the classical activating pathway; i.e., disorders resembling lupus and glomerulonephritis and asymptomatic patients have been recognized.

C1-INH

C1 inhibitor (C1-INH), a member of the SERPIN protease inhibitor family, binds to activated C1s, thereby irreversibly inhibiting the cleavage of C4 and C2. C1-INH is also involved, to a lesser extent, in the regulation of the coagulation, fibrinolytic, and kinin-forming systems [70]. C1-INH is a 104,000-dalton single-chain glycoprotein. Five laboratories have isolated and sequenced cDNA clones encoding C1-INH [71–75]. The mRNA of 1.8 Kb encodes a polypeptide of 478 amino acid residues (52,870 kDa) [71, 74]. The 17-Kb gene encoding C1-INH has been localized to chromosome 11, bands p11.2–q13 [71, 75]. Several polymorphisms in the C1-INH gene have been recognized using restriction fragment length polymorhisms; one is unrelated and the others are linked to the deficiency [76].

Deficiency of C1-INH is responsible for the clinical disorder termed hereditary angioneurotic edema (HANE). HANE, an autosomal dominant disease, is characterized by recurrent, acute, local circumscribed edema of the skin or mucosa. The primary sites involved are the extremities, face, larynx, and gastrointestinal tract [77–80]. C1-INH deficiency can be divided into two types [81–82]. In type I, which comprises 85% of affected kindred, a normal C1-INH protein is present in plasma in reduced concentrations (5% to 30% of normal). Type II is characterized by normal to elevated antigenic levels of C1-INH due to the presence of a dysfunctional mutant protein [83, 84]. Multiple mechanisms account for the phenotype of C1-INH deficiency. For example, in type II deficiency the abnormal C1-INH proteins differ functionally. [85]. That is, each of eight isolated dysfunctional proteins showed a different spectrum of inhibitory activity with C1s, plasma kallikrein, activated Hageman factor, and plasmin. Also, restriction fragment length polymorphisms have been associated with type I deficiency [76, 86].

Acquired C1-INH deficiency

Acquired C1-INH deficiency is characterized by the onset of angioedema accompanied by hypercatabolism, not a decrease in synthesis, of the protein [87, 88]. Most of the reported cases of acquired C1-INH deficiency are associated with benign or malignant B-cell lymphocytic leukemia, macroglobulinemia, multiple myeloma, and essential cryoglobulinemia. It is felt that in these cases immune complexes, consisting of anti-idiotypic antibodies to IgG expressed on malignant B lymphoma cells, bind and activate C1, resulting in consumption of C1-INH, which then leads to symptoms of angioedema [89]. A second type of acquired C1-INH deficiency results from development of autoantibodies to the C1-INH molecule [90, 92].

C2

The second complement component (C2), a 110,000-dalton single-chain protein, is the serine esterase component of the classical pathway C3 cleaving enzyme. A full-length C2 cDNA has been cloned and sequenced [42]. C2 mRNA (2.9 Kb) directs the synthesis of a 754-amino-acid primary translation product that yields a plasma form of C2 composed of 734 amino acids [42]. In addition, two smaller C2 polypeptides that remain cell-associated are synthesized [35, 93].

C2 is one of the complement components encoded by a gene located in the major histocompatibility complex (MHC) of man (HLA) and mouse (H2). These MHC class III complement genes are closely linked, and no cross-over has been detected among them so far. C2, together with the genes for factor B and two loci encoding C4, constitute the MHC class III genes [94]. The organization of the class III genes in man is diagrammed in figure 18-3. The C2 gene (18 Kb) is present as a single copy [95] upstream of the other class III genes <400 bp from the B gene. A *cis*-acting DNA sequence located within the 3′ noncoding region of the C2 gene apparently participates in regulating interleukin-1 (IL-1) mediated expression of the B gene [96].

Isoelectric focusing gels reveal four C2 polymorphisms [30]. The most common variant, designated C2C, has a frequency of 0.95 in Caucasian populations. Most individuals, therefore, are homozygous for C2C. A basic variant, C2B, occurs with a frequency of 0.04–0.05. Two rare acidic variants, C2A1 and C2A2, have also been detected. Two restriction fragment length polymorphisms of the C2 gene [95] represent genetic markers distinct from the protein polymorphisms and thus subdivide the C2C allotype [35].

Homozygous C2 deficiency is the most common inherited complement deficiency state in Western European populations. The minimum frequency for heterozygous C2 deficiency is approximately 1.2% [32, 97], and homozygous C2 deficiency occurs at a frequency of 0.01% [98]. Interestingly, C2 deficiency exhibits very strong linkage dysequilibrium with certain HLA haplotypes and complement polymorphisms. For example, the haplotype/complotype most characteristic of C2 deficiency is HLA-A25,B18,C2Q0,

BfS,C4A4,C4B2,D/DR2 [98]. Preliminary studies have been performed to ascertain the molecular mechanism accounting for C2 deficiency [99]. Employing a partial C2 cDNA as a probe, no major gene deletion or rearrangement could be detected, but no C2 mRNA was present in monocytes from homozygous deficients. These data suggest a transcriptional defect in C2 expression.

Many C2-deficient individuals present with clinical signs and symptoms of an SLE-like disorder, but some are asymptomatic. In other kindreds, association of C2 deficiency and recurrent pyogenic infection has been observed. Other rheumatic diseases described in C2-deficient individuals include glomerulonephritis [100], dermatomyositis [101], anaphylactoid purpura [102], and vasculitis [103], but the possibility of ascertainment bias cannot be ruled out in each instance.

Guinea pig model

In 1981, a genetic deficiency of C2 in guinea pigs was reported [104]. As in humans, the guinea pig C2 gene is localized within the MHC [104]. Biosynthetic studies demonstrate that macrophages isolated from these animals do not synthesize and secrete functionally active C2 [105], but a C2 protein with a slightly smaller size was detected intracellularly. Further studies done at the gene and mRNA levels should reveal the mechanism responsible for generating this abnormal nonsecretable protein. These animals should also prove to be an excellent model to examine the role of C2 deficiency in autoimmune diseases , since C2-deficient guinea pigs demonstrate a defect in antibody synthesis and isotype switching [106].

C3

The third component of complement, C3, is a 195-kDa glycoprotein that circulates in plasma as a disulfide-linked heterodimer. C3 is synthesized as a single-chain precursor, pro-C3. Postsynthetic cleavage of prepro-C3 by signal peptidase and a plasminlike enzyme generates the circulating native C3 [107]. Human [108], mouse [109, 110], and rabbit [111] C3 cDNAs have been cloned and sequenced. The corresponding mRNA encoding C3 is approximately 5.2 Kb in length [108]. The C3 gene has been localized to chromosome 19 in man and 17 in mouse [112–114]. The mouse C3 gene has been determined to be approximately 24 Kb in length [115].

Polymorphic variants of human C3 have been detected at the protein level [116]. C3-deficient patients all suffer from recurrent infections with pyogenic encapsulated bacteria. Of the 14 C3-deficient individuals thus far reported, one third also have nephritis, systemic lupus, cutaneous vasculitis, or proteinuria [24, 117]. Not much information has been obtained that might give insight regarding the molecular mechanism of C3 deficiency, but one investigation indicates that the rate of C3 synthesis by deficient patients' monocytes is 25% of that observed in monocytes from normals [118]. However, plasma con-

centrations of C3 in the homozygous deficients was less than 0.1% of normal. The basis for this finding needs further investigation.

Guinea pig and dog models

In 1983, the plasma of a strain of guinea pig designated Boulderwood (Bw) was noted to contain low (5.6% of normal) levels of C3 [119, 120]. As in the human C3 deficients, the rates of C3 synthesis and secretion in monocytes and hepatocytes are greater than would be expected from the serum levels and are, in fact, essentially normal. It has been suggested that the C3 that is synthesized is structurally abnormal and might be susceptible to the action of proteolytic enzymes [121]. Interestingly, the C3-deficient guinea pig has an impaired humoral immune response [120].

In 1981, a strain of Brittany spaniels, inbred to study the genetics of hereditary canine spinal muscular atrophy, was found to be C3 deficient [122]. The clinical manifestations of C3 deficiency in the dog are similar to those exhibited by C3-deficient humans. That is, a number of the C3-deficient dogs have had severe bacterial infections. Some of the dogs with C3 deficiency, like their human counterparts, have developed renal disease [123].

C4

The fourth complement component, C4, is a 200,000-dalton glycoprotein that circulates in the plasma as a heterotrimer. These polypeptide chains, designated α, β and γ, are held together by disulfide bonds and noncovalent forces. C4 is synthesized as a 185,000-dalton single-chain precursor, pro-C4 [124, 125]. Human [126] and mouse [127, 128] cDNAs that encode the pro-C4 molecule have been isolated and fully sequenced. These studies demonstrated that pro-C4 is synthesized from a 5.3-Kb mRNA.

C4 is encoded by two separate genes, C4A and C4B [56, 129]. These two genes are located approximately 10 Kb apart witha the MHC (see figure 18-3). The C4A gene is 22 Kb in length, but two sizes of the C4B gene have been detected, 16 Kb and 22 Kb [56, 130, 131]. The C4A and C4B genes both express mRNAs and protein products. However, the two C4 gene products vary in 1) electrophoretic mobility, 2) specific functional hemolytic activity (C4A and C4B bind preferentially to amino groups and hydroxyl groups, respectively), and 3) Rodgers (C4A) and Chido (C4B) serum- and red-cell-associated antigens. These differences in the C4A and C4B proteins are primarily the result of four amino acid substitutions located within the α-chains, 106 amino acids downstream of the thiolester site [35, 126]. In addition to these four amino acid substitutions, the C4A and C4B proteins are extremely polymorphic. In fact, differences in electrophoretic mobilities of neuraminidase-treated plasma [132], serologic typing [133], and direct DNA sequence analyses [126] suggest that there are altogether more than 40 alleles in the two classes.

Interestingly, the frequency of the *null* (nonfunctional) alleles C4A*Q0 and

C4B*Q0 is high in several populations [133, 134]. It has been suggested that this high frequency is due to unequal crossing over between very close genes, as for the α-genes of hemoglobin [135]. Unequal crossing over would lead to haplotypes with two expressed C4 genes, to one haplotype with one gene and a second haplotype with three genes. Duplications of the C4A and C4B genes, as well as *half-null haplotypes*, have been described [136, 137]. A substantial proportion, but not all C4 null loci, can be accounted for by gene deletion events so that other molecular defects may also result in C4 deficiency.

The frequency of half-null haplotypes has been estimated to be 16%–19% and 9.5%–14% for C4A*Q0 and C4B*Q0, respectively [138]. In several studies, a significant association of C4A*Q0 with SLE has been observed [137, 139, 140]. Both C4A*Q0 and C4B*Q0 have been associated with insulin-dependent diabetes mellitus [141, 142] and IgA nephropathy or anaphylactoid purpura [143]. Because of the linkage of the C4 genes to other genes of the major histocompatibility complex, it is not certain if the disease association is directly related to the absence of a particular C4 protein or another gene within the MHC. However, one report indicates that the risk of SLE is associated with C4A*Q0 and is independent of other loci within the MHC [144]. The recent recognition of differences in the response of the two C4 genes of interferon-γ-dependent regulation, coupled with the aforementioned functional differences in the gene products, suggests a possible direct role for these variants in the predisposition to SLE.

A C4 *null haplotype* C4AQ0BQ0 is a rare event; hence, only 17 cases of complete C4 deficiency have been reported in 12 families. Twelve of these complete C4-deficient individuals presented with lupus erythematosus. These patients have displayed many of the classical clinical features of SLE, such as photosensitive skin rashes, renal disease, and occasionally arthritis. However, only two individuals had antinative DNA antibodies. Therefore, in most cases of complete C4 deficiency, the clinical symptoms are that of those observed in *antinuclear antibody negative* SLE [138].

Guinea pig model

By chance, an outbred guinea pig colony was discovered to be deficient in the fourth complement component [145]. There is only a single C4 gene present in guinea pigs, and by Southern blot analysis it is clear that this gene was not deleted in the C4-deficient animal [146]. Northern blot analysis revealed an abnormally large (7-Kb) C4 mRNA expressed in liver of the C4-deficient strain [147], suggesting that the deficiency is due to a posttranscriptional defect in processing of the precursor RNA to mature C4 mRNA.

C5

The fifth complement component, C5, is a 200,000-dalton serum glycoprotein that is made up of two polypeptide chains, α and β [148]. C5 is synthesized as a 180,000-dalton single-chain precursor, pro-C5 [149]. Human [150, 151]

and mouse [152] cDNAs that encode all or part of the pro-C5 molecule have been isolated and sequenced. From these data, it was determined that the β-chain is the amino-terminus of the pro-C5 molecule and is therefore similar in organization to pro-C4 and pro-C3.

C5 is encoded by a single copy gene more than 50 Kb in length [153], located on the second chromosome in the mouse [154] and on the long arm of chromosome 9, bands q32–34, in humans [151]. The only polymorphisms of C5 thus far detected have been in populations in the South Pacific region [155]. With the availability of C5 cDNA probes it is now possible to examine C5 polymorphisms at the gene level.

Fifteen individuals with homozygous C5 deficiency have been reported [156]. Sera from these individuals lack bactericidal and chemotactic activities [157, 158]. These C5-deficient individuals, like others with deficiency of C6, C7, and C8, display a propensity for severe, recurrent infections particularly to Neisserial species, including meningitis and extragenital gonorrhea. Heterozygote-deficient individuals are asymptomatic.

Mouse model

C5 deficiency in the mouse was one of the first complement deficiencies discovered. The first strain characterized, B10 D2/oSn, was found in 1962 [159]. In 1964, Cinader et al. [160] showed that a serum protein, which they called MuB1, was absent from plasma of these mice, and later Nilsson and Muller-Eberhard [161] identified the missing protein as C5. Approximately 40% of commercially available inbred mice strains are C5 deficient. All C5-deficient strains examined thus far exhibit the same molecular defect. Studies of several different C5-deficient strains revealed that C5 deficients fail to secrete C5 protein, but biosynthesis of pro-C5 was detected [162]. In 1987, Wheat et al. [163] demonstrated the presence of polymorphisms in the C5 gene linked to the deficiency, and two forms of C5 mRNA in C5-deficient cells. However, the relationship of these polymorphisms to the genetic mechanism of the deficiency is not yet known. Recent isolation of the C5-sufficient and C5-deficient genes [153] will help in elucidating the exact molecular mechanism of C5 deficiency.

C6 AND C7

The sixth and seventh complement components are each single-chain glycoproteins of approximately 115,000 daltons. Genetic polymorphisms indicate that the two genes are closely linked, though the chromosomal location has not been determined [164]. A full-length C7 cDNA has recently been cloned and sequenced [165]. From these data it was determined that the C7 mRNA (3.9 Kb in length) directs synthesis of a pre-C7 molecule of 843 amino acids.

Individuals homozygous-deficient for C6 and C7 are predisposed to recurrent disseminated Neisserial infections [166]. With the isolation of C6

and C7 cDNA clones, it is now possible to examine at a molecular level the mechanism accounting for these deficiencies.

Rabbit model

There are three known strains of C6 deficient rabbits. The *Freiburg strain* was the first discovered [167]. Two additional strains have since been found, the *Mexico strain* [168] and the *Cambridge strain* [169]. However, very little is known about the molecular mechanisms leading to C6 deficiency in these animals, or whether each strain represents a different mutation.

C8

The eighth component of complement (C8) consists of three nonidentical subunits arranged as a disulfide-linked $\alpha-\gamma$ dimer (molecular mass Mr 88,000) and a noncovalently associated β chain (Mr 64,000) [170, 171]. The α and β chains show sequence homology to one another and to C9, the LDL receptor, and EGF. Studies of C8 polymorphisms established that $\alpha-\gamma$ and β subunits are encoded by separate genes that are closely linked on chromosome 1 [172, 173]. Recently, full-length cDNAs have been isolated for all three C8 chains [52–54]. By employing these specific cDNAs as probes in Northern analysis, it was demonstrated that the α, β and γ chains are encoded by separate mRNAs of 2.5, 2.6, and 1.0 kilobases, respectively [174].

Since three genes encode the C8 molecule, it is not surprising that the complexity of this defect approaches that of C1 deficiency. For example, C8 deficiencies can be organized into two groups: 1) deficiency of $\alpha-\gamma$ protein, and 2) absent β-chain protein [175, 176], or an abnormal nonfunctional β-chain protein [177].

Less than 50 C8-deficient patients have been reported [175], but nearly all exhibit increased susceptibility to infection with Neisseria. In rare cases, C8 deficiency has been associated with lupuslike syndrome [178, 179].

Rabbit model

A C8 $\alpha-\gamma$ deficiency had been identified in a strain of New Zealand White rabbits [180]. These C8-deficient rabbits have a lower survival rate for the first three months of life when compared to normal rabbits. In addition, their body weights were reduced by 25% on average. Some of the C8-deficient rabbits have recently been found to have low levels of C3 [181]. However, this phenomenon occurred independent of the C8 deficiency.

C9

The ninth complement component, C9, is a single-chain polypeptide of 71,000 daltons. C9 cDNA has been cloned by two laboratories [55, 182, 183], leading to information that C9 mRNA (2.4 Kb) encodes a protein of 537 amino acids that shows sequence homology with perforin, the analogue of C9 produced by cytolytic T lymphocytes [184]. Studies on the structure of the

human C9 gene indicate that the coding region is encoded by 12 exons covering at least 80 Kb [185].

C9 deficiency is relatively common among Japanese. Of 100,000 sera tested in Japan, 103 were found to be C9 deficient. Unlike the other terminal complement components, deficiency of C9 seems to carry little risk for clinical disease [186]. Only one individual C9-deficient patient in the United States has had problems with infection [187]. Therefore, C5b-8-mediated lysis seems to provide sufficient defense against Neisseria.

FACTOR I

Factor I, a serine protease consisting of two disulphide-linked polypeptide chains (46,000 and 39,000 daltons) cleaves fragments of C3 and C4 (C3b and C4b). Two polymorphic variants of factor I have been detected in Japanese [188]; however, no polymorphisms have been detected in other racial groups. Factor I cDNA has been cloned, and analysis indicates a polypeptide chain of 565 amino acids [189, 190], generated from an mRNA of 2.4 Kb [189]. The factor I gene has been localized to chromosome 4 [190].

Factor I deficiency has been reported in several kindred [166]. In these patients, C3 and other alternative pathway proteins are depleted because of uncontrolled activation of the amplification loop of the alternative pathway [191–193]. Factor-I-deficient patients suffer from recurrent infection with pyogenic encapsulated bacteria due to a relative deficiency of opsonic factors produced by wasteful consumption of C3 protein.

FACTOR H

Factor H is a single-chain plasma glycoprotein of 155,000 daltons that is a cofactor in regulation of C3b by factor I. Human and mouse factor H cDNAs have been isolated and sequenced [50, 194, 195]. From these studies, factor H was determined to be made up of a series of 20 60-amino-acid repeat structures similar to those in C4-binding protein and other proteins. Five variants of factor H protein have been reported. One of these polymorphisms results from the presence of either a tyrosine or histidine residue at position 384 [196].

At least two H-related mRNAs have been visualized [194, 197]. The mRNA corresponding to the 155,000 plasma protein is 4.3 Kb. In addition, a 1.8-Kb mRNA probably generated by alternative splicing appears to program translation of a 50,000-dalton H protein [194, 197]. An additional minor factor H mRNA of 1.3 Kb has also been detected in liver, and is recognized only by probes derived from the 3′ end of the H cDNA [194].

The gene for H has been localized to the long arm of chromosome 1 [198]. Preliminary analysis of the human gene suggests it is approximately 90–100 Kb in length, and that a related gene or pseudogene may also be present [199]. In the mouse, the H gene is about 90 Kb and at least two H-related genes or pseudogenes of 60 Kb and 120 Kb are also present [200].

Two cases of complete H deficiency in humans have been reported [201].

Plasma from these individuals, like factor I deficients, contains reduced levels of C3, factor B, and properdin because of continuous activation of the alternative pathway. One of the affected patients, an 8-month-old boy, had hemolytic uremic syndrome [201]. However, his brother, also H deficient, showed no sign of disease.

DECAY-ACCELERATING FACTOR (DAF)

Decay-accelerating factor or DAF is a glycoprotein of about 70,000 daltons that is present on the surface of a wide variety of cell types. Erythrocytes, all leukocytes, platelets, epithelial cells, and connective tissue cells contain DAF on their membrane surfaces. A soluble form is also present at low concentrations in plasma, tears, saliva, and urine [51, 198, 202]. DAF binds to the C3/C5 cleaving enzymes, accelerating their decay, thereby protecting bystander cells from complement-mediated lysis.

The cDNA encoding DAF has been isolated from permanent cell lines (HeLa and HL-60) [203, 204]. Different transcripts, perhaps derived from alternative splicing of the DAF gene, have been detected. Membrane-bound DAF is a 347-amino-acid, 70,000-dalton protein, heavily glycosylated, that is anchored to the membrane by a glycophospholipid moiety [203, 204]. The gene encoding DAF (35 Kb) is located on human chromosome 1, band q32 [205, 206].

Deficiency of DAF is associated with paroxysmal nocturnal hemoglobinuria (PNH), an acquired disorder [207, 208]. PNH is characterized by intermittent hemolytic anemia caused by complement-mediated autohemolysis. In these patients, DAF is also absent on granulocytes, monocytes, and platelets [209]. Furthermore, cells from PNH patients also lack other membrane proteins such as homologous restriction factor [210] and acetylcholinesterase [211], which are anchored by a glycophospholipid moiety.

COMPLEMENT RECEPTOR TYPE 1 (CR1)

Polymorphic variants of the complement receptor for C3b and C4b (Cr1) have been described. It is an approximately 200 kDa single-chain membrane glycoprotein found on erythrocytes, leukocytes, mast cells, and glomerular podocytes (reviewed in [212]). CR1 on red blood cells is a cofactor in factor-I-mediated cleavage of bound C3b and C3bi [212–214] and in the clearance of immune complexes (reviewed in [8]). CR1 also plays a role in histamine release by granulocytes [215].

CR1 cDNA clones have been isolated and sequenced [216], from which an estimated 78% of the coding region has now been ascertained. Three long homologous repeats, each of 450 amino acids, are present in the extracellular domain. Within each homologous repeat are seven consensus repeats of 60–70 amino acids each. Limited sequence analysis of CR1 genomic clones suggests that each repeat is encoded by a distinct exon, as is the case for the Ba fragment of factor B.

Several structural polymorphisms of CR1 have been identified. There allotypic variants, S, F, and F', are associated with proteins of different apparent molecular mass [217–220]. Each one of these polymorphic variants is encoded by a distinct mRNA, resulting from unequal crossing over, alternative RNA splicing of single heterogenous nuclear CR1 transcript, or generation of tandem repeats [221]. Another variant resulting in a lower number of CR1 molecules per cell, but without a change in structure [222] has recently been associated with a specific restriction fragment length polymorphism [223]. This variant is thought to be generated by a *cis*-acting regulatory element linked to and responsible for expression of CR1 on erythrocytes. The allele defined by this RFLP may be a disease susceptibility gene for systemic lupus erythematosus, though this point still needs additional work [224].

COMPLEMENT RECEPTOR TYPE 3 (CR3)

The complement receptor type 3 (CR3) binds the degradation product of C3b, iC3b. CR3 is present on monocytes, macrophages, granulocytes, polymorphonuclear neutrophils, and large granular lymphocytes. On unstimulated cells, CR3 is felt to play a supportive role in the function of other receptors, e.g., Fc-receptor, in mediating phagocytosis. However, when the cells are stimulated, the CR3-iC3b interaction is apparently sufficient to trigger phagocytosis [225, 226]. CR3 is comprised of two noncovalently linked subunits, α and β, with molecular weights of 155,000 and 95,000 daltons, respectively. CR3 is a member of a family of leukocyte membrane surface proteins that share an identical β-chain linked noncovalently to one of three distinct α-chains [226]. The other two members of this family are LFA-1, with an α-chain of 175,000 daltons, and p150,95, with an α-chain of 150,000 daltons. All are components of the CD11,18 complex. These represent a group of adhesion proteins that are part of a larger family of proteins (integrins). The α and β chains of CR3 are synthesized separately and then assembled prior to membrane insertion [227]. cDNA corresponding to the β chain of these adhesion proteins has been cloned and sequenced [228, 229]. The β chain is 769 amino acids long, including a 22-amino-acid signal peptide, a cysteine-rich external domain with 3–4 tandem duplications, a hydrophobic membrane-spanning region, and a 46-amino-acid cytoplasmic tail. The gene for the β subunit (~32 Kb) has been mapped to chromosome 21 [229–230]. Southern analysis of genomic DNA has revealed a single polymorphism present in the "normal" population, i.e., not linked to the deficiency [231].

Deficiency of this complex is referred to as leukocyte adhesion deficiency (LAD). The clinical syndrome includes severe bacterial infections, progressive periodontal deterioration, delayed separation of the umbilical cord, lack of pus formation, and leukocytosis. Leukocytes from these individuals are defective in adhesion-related functions including phagocytosis, cell-mediated cytolysis, binding to endothelial cells, and response to specific antigens. Studies with subunit-specific monoclonal antibodies indicate that cell surface expression of

the common β subunit and all three unique α subunits is deficient in leukocytes from these individuals. The defect is heterogeneous in that some are severely deficient, with less than 1% of normal CR3 expressed, and others are milder phenotypically, with 5%–10% of normal levels [232, 233]. Primary defects in the gene encoding the common β subunit acccounts for the deficiency in most of the reported cases [234–236]. Kishimoto et al. [237] have identified five distinct β-subunit abnormalities, namely, undetectable β-subunit mRNA and protein precursor; decrease in steady state levels of β-subunit mRNA; an aberrantly large precrsor; an aberrantly small precursor; and a normal-size precursor that does not undergop normal postranslational modification. In each case, the β subunit precursor does not associate with the α subunit. Limited restriction endonuclease digestion analysis of DNA from individuals lacking β-subunit mRNA does not indicate gross deletions or rearrangement in the β-subunit structural gene.

This chapter highlights an area of rapid expansion in the breadth and depth of information available. We trust that the molecular pathogenesis of these genetic deficiencies and potential therapies will result from ongoing work on this problem.

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