Ralph M. Trüeb
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Editors

Aging Hair

Springer

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Preface

"Aged? But he does not appear aged,
just look, his hair has remained young!"
Marcel Proust, In Search of Lost Time (1913–27)

The appearance of hair plays an important role in peoples' overall physical appearance and self-perception. With today's increasing life-expectation, the desire to look youthful plays a bigger role than ever. The hair care industry has become aware of this, and capable to deliver active products that are directed toward meeting this consumer demand. The discovery of pharmacological targets and the development of safe and effective drugs such as minoxidil and finasteride also indicate strategies of the drug industry for maintenance of healthy and beautiful hair in the young and old.

The study of hair aging focuses on two main streams of interest: On the one hand, the esthetic problem of aging hair and its management, in other words everything that happens outside the skin; on the other hand, the biological problem of aging hair, in terms of microscopic, biochemical, and molecular changes, in other words the "secret life" of the hair follicle in the depth of the skin.

Hair aging comprises hair shaft aging, and aging of the hair follicle. The former involves weathering and photoaging of the hair shaft, while the latter manifests as decrease of melanocyte function (graying) and decrease in hair production (alopecia). The scalp and hair are subject to intrinsic or physiologic aging, and extrinsic or premature aging due to external factors. Intrinsic factors are related to individual genetic and epigenetic mechanisms with interindividual variations. Prototypes are familial premature graying and androgenetic alopecia. Extrinsic factors include ultraviolet radiation, air pollution, smoking, and nutrition.

Finally, basic scientists interested in the biology of hair growth and pigmentation have exposed the hair follicle as a highly accessible and unique model that offers

unequaled opportunities also to the gerontologist for the study of age-related effects. Its complex multicell-type interaction system involving epithelium, mesenchyme, and neuroectoderm, and its unique cyclical activity of growth, regression, rest, and regrowth provides the investigator with a range of stem, differentiating, mitotic, and postmitotic terminally differentiated cells, including cells with variable susceptibility to apoptosis, for study. Ultimately, a number of intrinsic and extrinsic modulating factors for hair growth and pigmentation have been identified and are being further tested. Current lines of research and future directions for therapeutic interventions are gene polymorphism diagnostics, the hair follicular route for targeted delivery of active compounds affecting the hair, stem cells of hair follicular origin, and tissue engineering of the hair follicle.

This monograph attempts to provide an up-to-date overview regarding all aspects of hair aging. It includes in-depth contributions from internationally recognized experts on the biologic basis as well as on current concepts for the diagnosis, treatment, and prevention of hair aging.

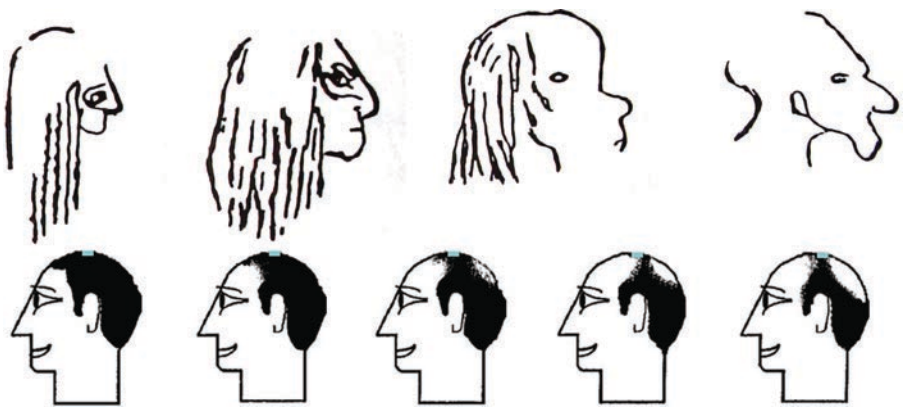
Zürich, Switzerland — Prof. Ralph M. Trüeb
Bradford, UK — Prof. Desmond J. Tobin

Foreword

Aging from Where to There?

Hair is part of the appearance of oneself as it is perceived by oneself and by others. The most remote representation that could be traced dates back around 30,000 years and the story is still ongoing in our society (Fig. 1; [1, 2]).

Figure reprocessed with permission from [1, 2]



In the early days, differences of hair patterns between species and between individuals within the same species (patterns, colors, length…) as well as dynamic changes of patterning (seasonal variations of hair coat, the fluctuations of hairiness during maturation…) reflect another scale of time and are part of a biological process that has been called "aging."

Human intervention has long been limited to representation, cutting, and sculpturing the mass of hair by physical removal of fibers. Some centuries ago, these aspects of hair care were exclusively privileged professional activities sometimes overlapping with medical/surgical practice. While styling and hair care modalities became – rather recently – part of personal care along with beauticians' and hairdressers' facilities, the biological and medical aspects became more and more part of the dermatological field of expertise, including all sciences associated with it (surgery, bioengineering, biology, biochemistry, physics, mathematics, etc.).

As hair and the hair follicle became a material for scientific observation, renewed interest is proposed in this book regarding the phenomenon of aging. Clearly, the arrow of time can be measured with various parameters. The exceptional regenerative properties of the hair follicle may lead to discoveries that are unsuspected by the scientific community as many keep a superficial understanding of the visible part of the iceberg: hair!

Let me give just one example taken from Bartholyn's book on anatomy. In 1658, hair was thought to be an excretory process for elimination of "bad bloody humors." One of the scientific arguments was that females after the arrest of menstruation grew beards. As those bloody humors had to find a way, the mechanistic interpretation was wrong, but it may still be considered as an appropriate clinical observation related to the field of endocrinology. Hence more recently better documented links were made between hormones (humors?) and the hair follicle productivity!

As usual, it took a long time between the accurate clinical observation and the proper understanding and scientific demonstration of a biological process underlying the expression of a clinical phenotype. It is to be hoped that this book will become a milestone to help anyone interested in hair and in aging leading to new avenues for a better understanding of the hair follicle biology during aging.

Top row of the figure shows four drawings were taken from wall engravings in a prehistorical cave. It took about 30,000 years in order to categorize patterned hair loss in males, shown in the bottom row. Most clinicians appreciate this as "progress" and use it daily in the hair clinic, but many agree that it is not sufficient when time-related changes are to be measured.

The arrow of time plays a major role in this chronic regressive process that affects the function and structure of the hair follicle. During the past 50 years and along with time, many steps involved in this process have been unravelled including but not limited to genetic predisposition, proper secretion of hormones, transport, metabolization, fixation on specific receptors, and translation in the cell nucleus of these hormones.

More research specifically devoted to aging will undoubtedly clear-up the hair-scene in the near future.

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Gerontobiology of the Hair Follicle

1

Desmond J. Tobin

Core Message

- The word "gerontology" is familiar to most of us as a term that captures the study of the social, psychological, and biological aspects of aging. However, its derivative "gerontobiology" as applied to the hair follicle is more concerned with the latter aspect – the biology of aging in the hair follicle mini-organ. As with any complex multicellular tissue system, the hair follicle is prone to broadly similar underlying processes that determine the functional longevity of organs and tissues. No matter how complex the tissue system is, it will contain cells that eventually lose functionality, reproductive potential and will ultimately die.
- The hair follicle is somewhat unusual among mammalian tissues in that it is a veritable histologic mélange of multiple cell types (e.g., epithelial, mesenchymal and neuro-ectodermal) that function contemporaneously in all stages of their life histories e.g., stem cells, transit-amplifying cells, and terminally differentiating cells. Some of these interactive cell systems appear to be nonessential for overall hair follicle survival (e.g., melanocytes). However, strikingly graying hair follicles may grow even more vigorously than their pigmented predecessors. Moreover, the hair follicle is unique in the adult mammal in that it follows a tightly regulated script of multiple lifelong cycles of cellular birth, proliferation, differentiation, and death. Powerful evolutionary selection ensures that the hair follicle is, in the main, hardwired against significant aging-related loss of function, even after 12 or more decades of life – although some would argue with this view, if only on purely cosmetic grounds.
- Processes underlying aging in general, e.g., oxidative damage, telomere shortening, age-relating deficiencies related to nuclear/mitochondrial DNA damage and repair as well as age-related reductions in the cells' energy supply, will all impact on whether some follicular cell subpopulations will enter cellular senescence. This chapter will focus on how gerontobiology of the hair follicle may impact on certain aspects of hair fiber phenotype.

1.1 Introduction

The function of scalp hair for humans is invested mostly in its value as a communication device or signal, and so together with epidermal pigmentation the hair fiber-producing mini-organ accounts for most of the phenotypic variation between different human subpopulations. Nature has made an enormous investment in the hair follicle, and as one of only two

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uniquely mammalian traits (in addition to mammary glands) serves several important functions for most other mammals. These include thermal insulation, camouflage, social and sexual communication (involving visual stimuli, odorant dispersal etc.), sensory perception, and protection against trauma, noxious insults, insects, etc. Because of our relative nakedness most attention and study is focused on scalp hair that, uniquely amongst primates, can be very thick, very long, and very pigmented. Thus, it is not surprising that its absence especially from the human scalp can result in significant psychologic trauma [14], e.g., in cases of androgenetic alopecia, alopecia areata, and chemotherapy-induced alopecia. Our ancient psychologic preoccupation with hair is further heightened today as our increasing longevity inevitably fuels our desire to extend youthfulness, where hair fiber density, texture, length and color all drive the unremitting growth of the hair-care market, already a multibillion euro enterprise worldwide (*Euromonitor*).

Human hair growth can be distinguished from that in most other mammals by its rather mosaic pattern of hair follicle activity; we have all but lost our ability to grow hair synchronously or as a wave. Instead, each hair follicle has significant autonomy for growth and pigmentation. The hair bulb exhibits the body's second highest rate of cellular proliferation (after hematopoietic and intestine tissue), and can still produce functional fibers right up until our last and oldest days of life – even if this extends beyond 12 decades.

1.2 Biologic vs. Chronologic Aging

Although humans have between 3 and 5 million individual hair follicles on their bodies, most attention be it academic or commercial is focused on the paltry 2% of these that are distributed on our scalps [37]. In fact, our "naked ape" moniker derives from the fact that the great majority of the other 4.9 million hair follicles produce only the finest of hair fibers. This diversity reflects, in part, the enormous differences in the time interval which hair follicles from different body sites spend in the growing phase of the hair growth cycle. The finest and shortest hairs spend only days to a few weeks in the anagen growth phase, while some scalp hairs can grow continuously for up to three decades to produce hair fibers of more than 4.6 m in length – all in a single although highly extended anagen phase! This biological variety of hair growth pattern complicates any discussion of hair follicle gerontobiology, as it draws immediate focus onto the hair follicle's relative proliferative and regenerative potential invested largely in its complement of stem cells. In addition, the local environment of the hair follicle can influence its behavior, and here too the human body exhibits considerable variation. Take for example hair follicle density in different body regions. An approximation for an adult Caucasian is: adult cheek skin (880 ± 60 HF per cm^2); forehead: 770 ± 60 HF per cm^2); forearm: 100 ± 50 HF per cm^2); upper arm: 40 ± 10 HF per cm^2) [12]. Thus the cheek contains more than 20 times more hair follicles per unit area that the upper arm.

Against a backdrop of such hair growth diversity are the additional contributions made by race and ethnicity, sex, climate and season, hormonal status, nutrition etc. One needs to take these factors into consideration before deducing underlying abnormality or before conflating biologic and chronologic aging factors. For example, the observation that males castrated before puberty do not go bald (does this impact on hair senescence?) or grow beards (does this impact on hair follicle maturity?) and the subsequent confirmatory finding that these individuals did so upon treatment with testosterone, indicates a role of androgens in hair growth [15]. But do these changes really reflect aging in action, either accelerated or retarded?

1.3 Variation in Hair Types During the Life of a Hair Follicle

The hair follicle produces several different types of hair fiber during a normal lifetime; represented by fine unpigmented lanugo hair in the fetus/neonate, short (mostly unpigmented) vellus hair or fine pigmented intermediate hairs during childhood, and long thick terminal hair shafts in several body sites in the adult. It is worth emphasizing that such modulation of hair fiber form from the relatively long, downy and variably pigmented hairs (lanugo) before birth to almost imperceptible colorless fibers (vellus) after birth, to coarse terminal hairs of phenomenal length (up to 4 m) all appear to occur within the same single hair follicle. Indeed, terminal hairs can be as abrasive as copper wire at certain cut lengths. This is, in my view, even more impressive than the striking range of hair types

characteristic of other species, e.g., vibrissae, tylotrich, zigzag, auchenes of mouse skin. In the latter mammal current data suggest murine hairs are each produced within their predetermined hair follicle subtypes [8]. Moreover, there is much clinical evidence of transformations between different forms of hair fiber produced by a single human follicle during the life of an individual in both health and disease.

1.3.1 Lanugo-to-Vellus Hair Follicle Transformation

Even before we are born our hair follicles have already produced two different types of hair fiber *in utero*. Lanugo hair is the first of these (produced during month 3–7) and is characterized by long pigmented unmedullated and silky hair. The exact function of lanugo hair during intrauterine life is unclear, though it is possible that this is related at least in part with the production of the vernix caseosa and additionally may be involved in the removal of toxic substances from the developing fetus – as this rapidly growing skin tissue produces millions of hair fibers that are usually shed *en masse* in a synchronized manner around month 7–8 of gestation. Thereafter these same follicles engage in the production of a much finer, shorter, variably medullated and less-pigmented hair fibers (so-called vellus hair), which are also shed *en masse* usually during the fourth month of extrauterine life. Before this second "molt" the entire surface of the neonate, with the exception of the scalp and eyebrows, is covered with short and very fine unpigmented vellus hairs. The third generation of hair to be produced by hair follicles in humans switches to a more mosaic pattern of hair cycling, where significant autonomy is invested in the individual hair follicle. It should be noted here however, that partial resynchronization of human hair follicles can be induced later by systemic extra-follicular stimulation including via endocrine factors etc. at different stages of life e.g., during pregnancy [23].

Hair fiber form can also to revert from vellus to lanugo in the adult. For example, acquired hypertrichosis with lanugo-like hair may be associated with an underlying neoplasm (mainly lung and colorectal cancer), so-called paraneoplastic hypertrichosis lanuginosa acquisita [4, 31]. Furthermore, reversion to lanugo-like body hair is one of several dermatologic signs in patients with eating disorders [34]. These cases highlight the extreme difficulty in applying general gerontobiologic scenarios to hair follicle aging, as the hair follicle appears to be able to "reinvent" itself regardless of the chronologic age of the individual. Congenital universal hypertrichosis (e.g., Ambras syndrome) represents a rather dramatic human phenotype where the sequence of hair type usually associated with normal development and growth is disrupted.

1.3.1.1 Congenital Universal Hypertrichosis

Recently interest in the life history of single hair follicles has focused on changes that occur in Ambras syndrome and other congenital hypertrichotic conditions. These have illuminated how the early hair follicle, formed during embryogenesis in the womb, may fail to correctly follow the usual transformational script from lanugo to vellus hair during the transition from intrauterine to extrauterine life. In this syndrome there appears to be a block on the normal shedding of lanugo hair from the hair follicle at around 7 months of gestational age [26]. Instead the child grows up with facial and body hair that can be very long and pigmented, although still mostly silky in texture. Affected individuals may have additional minor facial anomalies (dimorphism) or abnormalities of teeth, e.g., slower or even absent dentition. A further interesting feature of this disorder is the absence of any endocrine/hormone abnormality. The syndrome was first described in 1993 by Baumeister [2] and its multiple affected relatives suggest a genetic basis. Studies have recently implicated a chromosomal anomaly involving a breakpoint defect in the q22 region of chromosome 8. These data suggest that this region on chromosome 8 contains a gene that is disrupted in Ambras syndrome. Cases that have received cytogenetic analysis have shown at least a chromosomal inversion, though others additionally show an insertion and a deletion in addition to the inversion [11]. There remains some debate regarding the exact nature of genetic alteration required for a diagnosis of Ambras syndrome.

For most individuals however, the dramatic age-related change in hair form (excluding loss of hair color and loss of the hair itself) results from so-called hair follicle transformations after birth [17, 32, 36].

1.3.2 Vellus-to-Terminal Hair Follicle Transformation

Hormonal stimulation of vellus hair is known to drive vellus-to-terminal hair transformation in skin with secondary sexual characteristics (e.g., pubic, beard, axillary etc.) during puberty, and in hirsutism and hypertrichosis. The reverse transition from terminal-to-vellus is characteristic of androgenetic alopecia or male-pattern baldness [44]. These transformations can be remarkably rapid, classically evidenced by the puberty-associated changes in hair phenotype. However, other clinical evidence shows that sex steroids are not the only inducer of this change. For example, the reversal of the terminal-to-vellus transformations in finasteride- or minoxidil-stimulated hair follicles over a single hair cycle [44] does not appear to involve modulation of androgen action in any way, but indicates that the hair follicle itself remains susceptible to significant reprogramming in terms of fiber output.

Both routes to terminal hair transformation are likely to involve significant plasticity of the follicular papilla and dermal sheath [18], and alterations in follicular papilla cell number lie at the heart of any attempt to explain clinically important increases and decreases in hair fiber size. The hair follicle mesenchyme was long believed to consist of very stable fibroblastic cell populations. However, recent murine data indicate that follicular papilla cell number actually increases during early anagen and that this increase is driven primarily by cell proliferation in the proximal dermal sheath, followed by immigration of progeny cells into the follicular papilla [38]. Hypertrichosis (itself a form of vellus-to-terminal hair transformation) can also occur in certain body sites of both men and women of advancing age e.g., terminal hair growth on the upper lip and chin of postmenopausal women and on the ear pinae, nose, and nasal vestibules in aging men [43].

1.3.3 Terminal-to-Vellus Hair Follicle Transformation

It is not yet clear whether the terminal-to-vellus hair follicle transformation, most visibly manifested in androgenetic alopecia in males with age, is indeed simply a reversal of the earlier observed vellus-to-terminal hair follicle transition [10, 38, 39]. Several studies have helped to form a consensus that hair follicle miniturization with age is most likely to occur via relatively abrupt reductions in follicular papilla and/or dermal sheath cell numbers both during and between individual hair cycles. This contrasts markedly with the previously dominant view that hair shaft miniaturization, in male-patterned alopecia at least, occurs via a slow and gradual cycle-by-cycle change. There is convincing clinical support for the "abrupt change" view however, not least via the rapid progression of male-patterned alopecia and the preponderance of fine hairs over intermediate hairs in balding scalp [44]. Similarly, the reversal of the vellus-to-terminal transformations in finasteride- or minoxidil-stimulated hair follicles over a single hair cycle in this type of alopecia [44] supports this view.

In addition to hair follicle transformations to vellus or *invisible* hairs (the usual clinical appearance of "hair loss") there may also be a reduction in the absolute numbers of hair follicles, not only in the scalp but also throughout the body. The precise mechanism for this low-level hair follicle dropout is unclear, though it may mimic the programmed hair follicle organ deletion that can occur in mice with age [9]. Atrophic change and fibrosis can also be found in aging skin [25].

1.4 Age-Related Hair Growth Variation

In addition to the aforementioned significant variation in hair form during the extent of a normal human lifespan, hair growth rates also vary significantly during human aging and for different body sites. Indeed, when these are averaged for post-40-year old nonbalding males, hair actually grows most rapidly and with greater individual fiber thickness in certain body sites in individuals during their 50–70 years of age [25]. Increasing age can leave its mark on several phenotypic properties of the hair fiber.

While the most visually apparent of these include hair thinning, hair loss, reduction in the rate of growth, pigmentation loss [7, 19, 24, 40], aging can also affect change in the surface morphology of hair. This can be seen for both a reduction in the cuticular scale size, as well as loss of hair fiber lubrication/moisturization. Loss of hair shaft moisture and lubrication has been reported to occur as a function of increasing age in adults, especially in women. The hair exists within the context of the "pilo-sebaceous unit" – a term that

implicates the sebum-producing gland in several aspects of hair biology. The open and interactive nature of the pilo-sebaceous unit is facilitated by a duct that carries sebum from this holocrine tissue directly onto the hair fiber and from there to the skin surface. The activity of the sebaceous gland changes dramatically as a function of gender and age, from the relatively inactive prepubertal period, through to a very active adolescent and young adulthood, to markedly reduced activity after the fourth decade of age, especially in females [45]. In addition to crude overall changes in gland size and activity, more subtle changes involve modification of the composition of lipids being produced by this gland at different times during our lives. For example, sebum from children contains less squalene and cholesterol than sebum from adults [33].

Moreover, the concentrations of integral cholesterol sulphate and cholesterol have also been examined in human scalp hair shafts in 50 subjects, aged 18–87 years [3] to determine whether aging influences the integrity of the cell membrane complex which mediates cortical cell-to-cell cohesion in fully keratinized hair. A small but statistically significant increase with donor age was detected for hair cholesterol (but not for cholesterol sulphate), and was speculated to reflect changes in keratinization with age. The potential of this finding as a biomarker of aging was discussed by the authors of this study.

Aging is also associated with a reduction in the duration of active hair growth and in the diameter of hair shafts, which can be seen most readily in large caliber hair shafts. However, there can also be increased irregularity of the outline of the fiber, including increase angularity of the fiber's cross-sectional profile. There is also lengthening of the duration of the kenogen interval of the hair growth cycle i.e., the period after exogen and before the emergence of new anagen hair [27]. These changes resemble those observed in the course of male-pattern balding, although their development is less marked [7]. The perception of changes in hair density and overall hair volume can be modified by contemporaneous changes in hair pigmentation. Thus, while miniaturization of terminal hair during androgenetic alopecia does not appear to be associated with either previous or simultaneous loss of pigmentation in the affected hair follicles, canities can at least for faired-skinned and dark-haired individuals mask some of the more dramatic visual effects of hair thinning.

Recently there have been attempts to characterize changes to the internal structure of hair fibers with age [22, 29]. In one study Raman spectroscopy was used to compare the scalp hair fibers of Japanese females in their twenties with those in their fifties. This study found that the cystine disulfide (–SS–) content of the hair cortex decreased somewhat with age [22]. More recently, researchers analyzed the hair of so-called "anagen-blocked" scalp hair follicles [29]. The advantage of examining this particular case (Mrs YD – a 42-year-old Chinese woman) was their avoidance of any chemical processing and their protection of the hair from external weathering elements. In this way an assessment of natural (intrinsic) aging has been possible and this was characterized by a progressive abrasion to the cuticle from root to tip over the hair fibers, which were growing continually for over 26 years. This cuticular damage was further associated with a reduction in ceramides and 18-methyl eicosanoic acid and also in particular keratin-associated protein subfamilies (ultra-high sulfur proteins; high sulfur proteins; high glycine-tyrosine proteins). There was also a progressive decrease in mechanical resistance along these extremely long hair fibers.

1.5 Age-Related Hair Pigmentation Variations

Hair color in children tends to darken with advancing age [1] and it is not unusual for a blond child to be dark-haired even before the onset of puberty. Similarly, the phenomenon of heterochromia is much more apparent after puberty, with color differences between scalp and beard not uncommon. The fine scalp hair of the growing child and adolescent exhibits striking changes with increasing age to mature adulthood, not only in color (most typically a darkening of hair color e.g., blond to brown) but also by showing a coarsening of the hair fibers themselves. A reduction in the level of pigmentation of scalp hair in male-pattern baldness is associated with the reduction in the caliber of these hairs (see Chap. 9 elsewhere in this volume). This is thought to be largely the result of the reduced capacity of smaller, finer hairs to accommodate large numbers of melanocytes. There is also a tendency for these "miniaturizing" hairs to be less medullated than terminal scalp hair. By contrast, the loss of melanocytes from hair follicles producing hair fibers of normal

caliber (i.e., during hair graying or canities) may also result in a concomitant change in the structure of these hair fibers (see below). This is perhaps not surprising given the close interaction between melanin granule-transferring melanocytes and hair shaft-forming/melanin-accepting precortical keratinocytes.

It is likely that pigment-producing melanocytes in the hair bulb influence cortical keratinocyte behavior in several ways. For example, melanin transfer to cortical keratinocytes may hasten their terminal differentiation and cornification – a change may be mediated by increased levels of calcium, some of which may be transferred into the keratinocytes within melanin granules. For further discussion of the pigmentary changes associated with hair aging please refer to Chap. X–Z. Briefly, there is evidence that gray and white hair fibers exhibit different mechanical properties compared to adjacent pigmented hairs. Hollfelder provided some evidence that pigmented-free hairs are not only coarser but also can be wavier than pigmented hairs [16]. Moreover, others have reported that the average diameter of newly white hair fibers is significantly greater than that of pigmented hairs [40, 41]. Development of a more prominent medulla in white, compared to pigmented, hair fibers has also been reported in this study [40, 41]. Interestingly, these researchers have also described an age-related reduction in hair growth rate and in hair fiber diameter, but that this was broadly limited to pigmented hairs in these individuals. Thus, the implication is that, counter-intuitively, the apparently more "aged" white hairs may be partially spared some aging changes. The tensile strength of hair also decreases with age, having increased from birth to the second decade [7, 24]. A study by Van Neste on scalp hair growth in young, mature and menopausal women reported that the growth rate of non-pigmented hair in menopausal woman was higher than that of pigmented hair [42]. This difference remained statistically significant, when hair thickness (an important parameter of hair growth) was taken into account. Also, white hair was thicker on average, showed more medulla and grew faster than pigmented hair. However, the unpigmented hair of menopausal women grew at the same rate when compared with similar hair from younger women. Similar studies in scalp hair of younger and aging males are yet to be performed. The biology underlying these events requires further investigation, particularly in terms of observed regional variability as well as the potential influence of androgens or other hormonal factors involved [16, 29].

In a manner similar to the changes in lipid composition of sebum in individuals with advancing age, there is also an age-associated change in the chemical composition in the hair fiber. Metals that show this change in hair fibers include cadmium, copper, zinc and strontium [13]. Furthermore, reductions in glutathione reductase, glutathione-*S*-transferase, glucose-6-phosphate dehydrogenase and gamma-glutamyl transpeptidase have been reported [5, 6, 20, 35].

1.6 Hair Loss: An Aging Event?

Hair loss in men and women during aging is clearly a common event. However, the specificity of the finding in association with aging has been questioned. There have been anecdotal reports on so-called "senescent" alopecia [21], but the associated changes such as inflammatory infiltrates and fibrosis cannot be used to readily differentiate this type of alopecia from androgenetic alopecia [28, 30]. Independently of aging, hair color changes are suggestive but not specific for aging [21, 30]. The scalp is subject to both intrinsic (physiologic) aging and extrinsic aging caused by external factors. Intrinsic factors are related to individual genetic and epigenetic mechanisms and so show significant interindividual or interclan variation. Self-evident examples include familial premature graying and androgenetic alopecia. By contrast, extrinsic factors implicated in skin and hair aging include; ultraviolet radiation and smoking. Experimental evidence supports the hypothesis that oxidative stress plays a role in both skin and hair aging.

1.7 Conclusions

Aging of the hair follicle has traditionally been viewed in a rather simplistic and bipartite manner – namely alopecia and canities, and this has almost exclusively been limited to the scalp. This view may be too simplistic, as androgenetic alopecia can be already well-advanced in young scalps and premature graying does not appear to be linked to true chronologic aging of the general tissues systems of the affected individual. True hair follicle aging is instead likely to involve a much more subtle sequence of events, e.g., hair fiber cross-sectional

changes, which may be reversible (at least temporarily) as seen with restimulation of the melanocyte stem compartment after radiation of canities-affected scalp or the vellus-to-terminal hair follicle transformation upon successful finasteride or minoxidil treatment. Our increasing longevity has revealed that vigorous hair growth can continue for 120 years or more. Moreover, death of the individual (i.e., via other/multiple organ system failure) cuts short our view of the true life capacity of the hair follicle. In this context the hair follicle may be the best aging tissue of the body's complex tissue systems. Still Werner syndrome may curb this enthusiasm somewhat, as this model of human aging exhibits both early graying of the hair, and alopecia. However, "normal" aging of the hair follicle is unlikely to be dependent, like Werner syndrome, on a single defective gene product. Thus, much of the mystery awaits investigation.

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Molecular Basis of Androgenetic Alopecia

2

Valerie Anne Randall

Core Messages

- Androgenetic alopecia is a common, progressive, patterned loss of visible scalp hair.
- It causes psychological distress and negative effects on the quality of life.
- Adult levels of circulating androgens and functional intracellular receptors are essential prerequisites for the balding processes.
- Metabolism of circulating androgens, such as testosterone, to the more potent androgen, 5α-dihydrotestosterone, also appears necessary. This also acts via the androgen receptor.
- The current model for androgen action in the hair follicle focusses on the mesenchyme-derived, regulatory dermal papilla at the base of the follicle. This responds to the circulating hormones and coordinates the rest of the follicular cells by altering the paracrine signals it produces. These may be soluble growth factors or extracellular matrix components.
- There is also a strong genetic aspect to the incidence of androgenetic alopecia.
- Since the response to androgens occurs within the follicle, it can differ. Androgen responses vary from stimulation (e.g. beard), no effect (e.g. eyelashes) to inhibition on areas of the scalp (androgenetic alopecia). This paradoxical difference underpins the successful transplant of unaffected follicles from non-balding regions into the androgen-sensitive, balding areas during corrective surgery for androgenetic alopecia.

2.1 Introduction

Androgenetic alopecia, the most common form of hair loss in men, involves the progressive loss of visible, pigmented terminal hair on the scalp, in response to circulating androgens. It may also occur in women. Other names include: male pattern baldness, common baldness, male pattern alopecia, androgen-dependent alopecia, androgenetic alopecia or simply "balding". There are several other causes of hair loss such as the patchy baldness of the scalp and/or body of alopecia areata, generally believed to be an autoimmune disease [24]. These fall outside the scope of this book, but have been described elsewhere [10, 24, 94].

2.1.1 Patterns of Hair Loss

2.1.1.1 In Men

In men with androgenetic alopecia, the gradual replacement of long, pigmented, terminal hairs on the scalp

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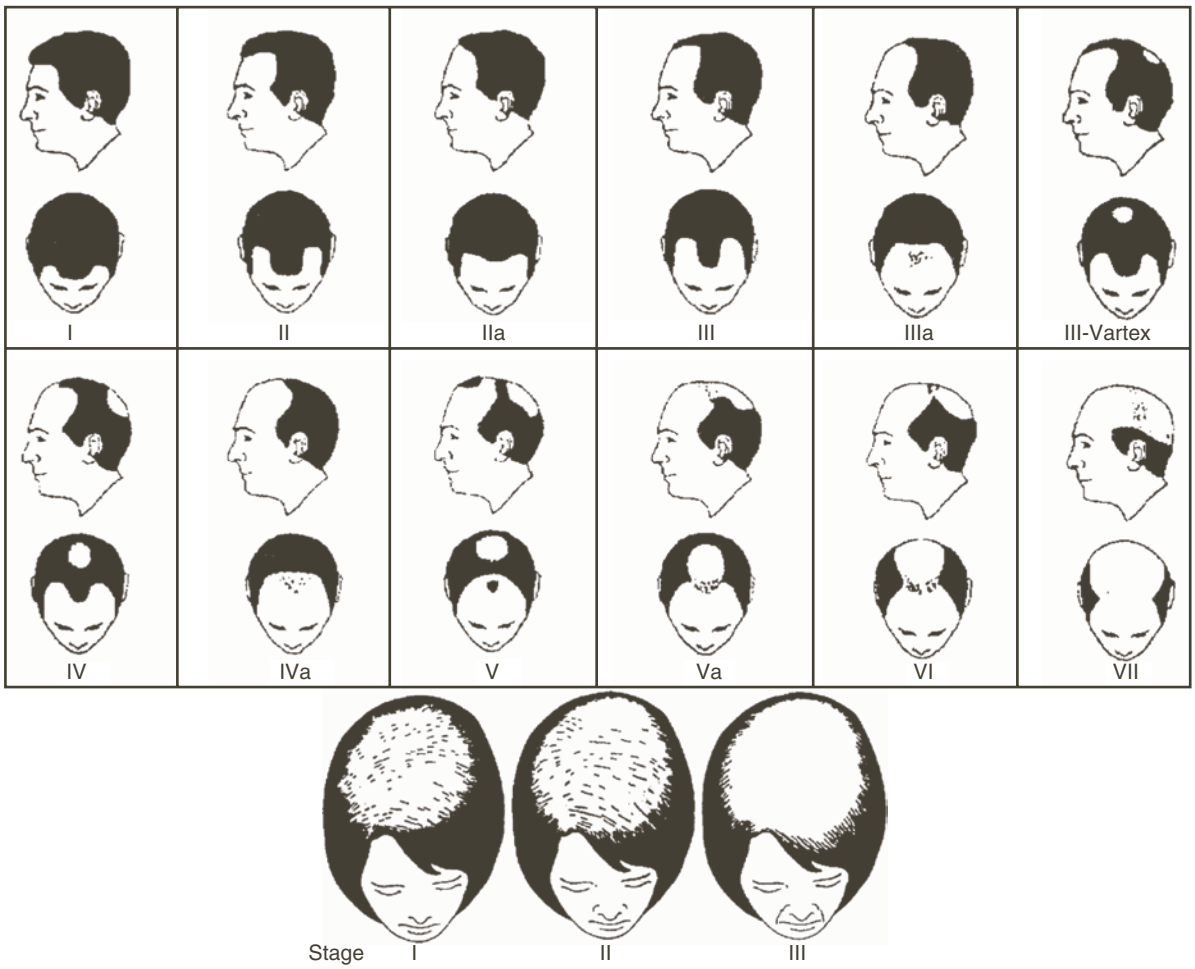

Fig. 2.1 Patterns of hair loss in androgenetic alopecia in men (*upper diagram*) and women (*lower diagram*). Androgens cause a gradual inhibition of hair growth on the scalp in genetically pre-disposed individuals. This is much more common in men than in women, and the pattern of the hair loss in men differs from women. In men, the first signs are generally temporal regression, which spreads backwards and joins thinning regions on the vertex to give a bald crown. In women, the front hairline is normally retained, and a general thinning on the vertex gradually becomes more pronounced until the vertex becomes bald (after Hamilton [4] and Ludwig [67])

by short, pale, *vellus* hairs normally occurs in a relatively precise pattern (Fig. 2.1). Hamilton graded this progression from type 1, pre-pubertal scalp with terminal hair on the forehead and all over the scalp, through gradual regression of the frontal hairline and thinning on the vertex, to type VII where the bald areas became fully coalesced to leave hair only around the back and sides of the head [51]. Norwood modified Hamilton's classification, including variations for the middle grades (see Fig. 2.1); this scale is used extensively during clinical trials [89].

2.1.1.2 In Women

Androgenetic alopecia is also reported in women, although androgen involvement is less established. Hamilton found post-pubertal recession to type II was common in Caucasian women with approximately 25% exhibiting the type IV pattern by age 50, although this did not develop further [51]. Although women can exhibit the "male" pattern, they usually show a different Ludwig pattern involving a progressive diffuse loss of hair from the crown while retaining the frontal hair line

(see Fig. 2.1) [80]. Venning and Dawber [137] found that 80% of pre-menopausal women had thinning in Ludwig stages I–III, while 13% had Hamilton types II–IV [7]; post-menopausally 37% exhibited the "male" pattern with some showing marked templar M-shaped recession, although not progressing beyond Hamilton stage IV.

2.1.2 Incidence

Although there are no precise statistics, the incidence in Caucasians is often quoted as approaching 100% [24]; others suggest that about half of men and women above 40 exhibit androgenetic alopecia [93]. There is a marked variation in other races, which often show much less balding. Most Chinese retain the pre-pubertal hairline after puberty, and baldness is less common, less extensive and starts later [51]. Japanese men also show a lower incidence, beginning balding about 10 years later than Caucasians [130]. Four times as many African-Americans also retain a full head of hair than Caucasians [121]. The reason for this racial variation is unclear, but is probably genetic because differences appear to be retained regardless of location.

2.1.3 Significance of Androgenetic Alopecia

Androgenetic alopecia is also seen in other primates, including the orangutan, chimpanzee and stump-tailed macaque [135]. This suggests a natural progression of a secondary sexual characteristic rather than a disease. In the past, when many men died young, marked androgenetic alopecia would have distinguished the surviving older male as a leader, like the silver-backed older male gorilla and larger antlers on older deer. Others have speculated that the bald patch of an angry older dominant male would flush and look very aggressive [43] or help in fighting because there was less hair to pull [28]. Whatever the potential benefit, the reduced incidence of baldness in African men [121] suggests evolutionary pressure to retain scalp hair for protection from strong sunlight.

Although androgenetic alopecia is common and neither life-threatening nor painful, it is a distressing disorder; Egyptian men's anxieties were recorded 4,000 years ago [40]! This reflects the important, although often underappreciated, roles of hair in human social and sexual communication, whatever the genetic background or culture. For example, the ritual head shaving of Christian and Buddhist monks and the short soldier haircuts are all designed to reduce individuality; these contrast with the religiously un-cut hair of Sikhs. In the youth-orientated culture of the industrialised nations, balding's association with ageing has very negative connotations and androgenetic alopecia often causes marked psychological distress and reduction in the quality of life in men [12, 36, 42, 81, 131, 139] and women [13, 136]. Patients report poor self-image, feelings of being older and loss of self-confidence. Similarly, other people report men with visible hair loss as older, less attractive, weaker and duller. Importantly, the same results were obtained in those who had never sought treatment [42]. Whatever may be its original biological role, androgenetic alopecia reduces the quality of life in the current industrialised world.

2.2 Changes During Androgenetic Alopecia

2.2.1 Altering the Type of Hair Produced Via the Hair Follicle Growth Cycle

The progressive loss of visible hair during patterned balding results from the gradual transformation of terminal follicles, producing the long, thick, pigmented hairs of youth, to smaller vellus follicles forming short, colourless, virtually invisible vellus hairs. This is a major change in cell biological terms; follicles possess a unique mechanism, the hair follicle growth cycle, which allows these changes [27, 73]. Each follicle normally undergoes a continual series of active, growing phases called *anagen*, alternating with periods of rest or *telogen*; these are separated at the end of anagen by a brief regression or *catagen* phase [27, 73] (see Fig. 2.2). This involves the destruction of the original lower follicle, and its total regeneration to form another follicle that can produce a totally new hair. The original hair is lost via active shedding called exogen [128]. In this way, the post-natal hair follicle appears to retain the ability to recapitulate the later stages of follicular embryogenesis throughout life.

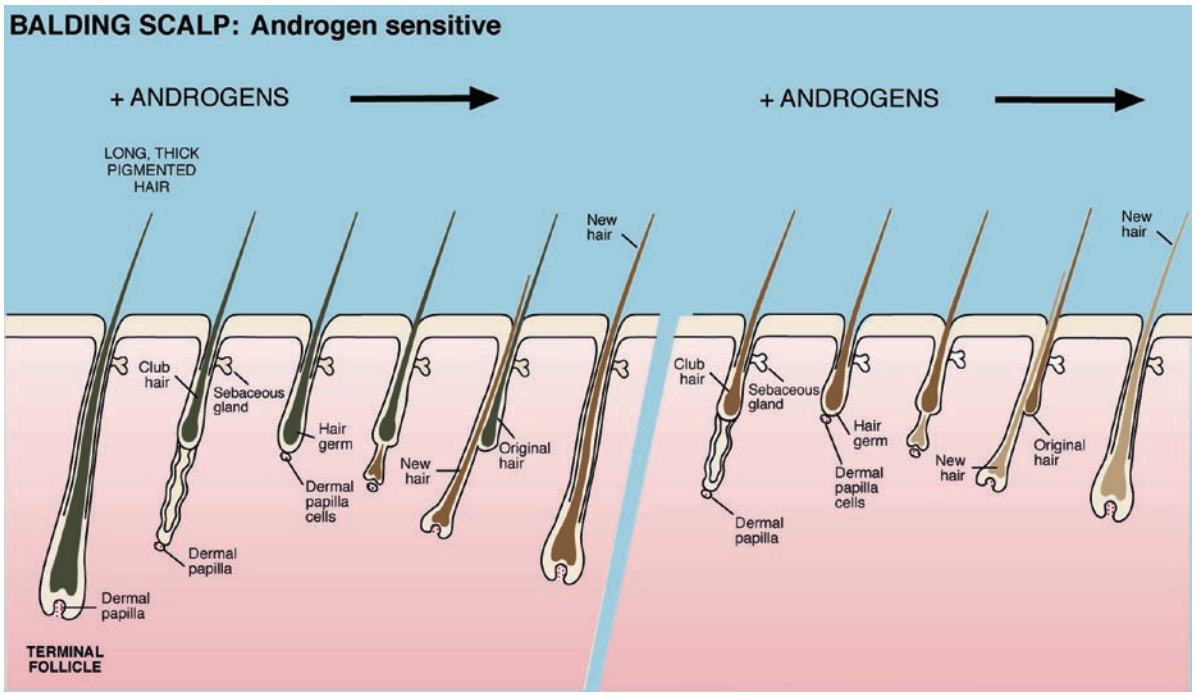

Fig. 2.2 Diagram representing the miniaturisation processes occurring in response to androgens in the scalp of a person with a genetic pre-disposition to androgenetic alopecia. Androgens inhibit scalp hair follicles in balding regions by inducing them to produce progressively smaller, finer and less pigmented hairs, until the terminal hairs of childhood and early adulthood are replaced by the vellus hairs of androgenetic alopecia and the area appears bald. Follicles themselves become shorter and finer, but must pass through a full hair cycle, probably a succession of cycles, to accomplish major changes. The white gap between the two parts of the diagram represents a space for at least one other cycle between the two shown to accommodate these changes in size

Many follicles will produce a new hair that is similar to the previous one, but the hair may differ in colour or size. It is unclear how much a hair can alter in size from the previous one, because many changes take place over several years e.g. developing a full beard [52]. The miniaturisation processes of androgenetic alopecia occur over many years with hair follicles reducing in size and depth in the skin and producing smaller and paler hairs (Fig. 2.2) [30, 119]. The type of hair produced by a follicle, particularly its length, depends greatly on the length of anagen. For example, long scalp hairs are produced by follicles with growing periods of more than 3 years [73, 119], whereas on the finger anagen may be only 1.5–3 months [119]. The cell biology and biochemistry of the local interactions involved in the control processes of the hair cycle are complex and not yet fully understood, but the size and length of the hair is controlled by the mesenchyme-derived dermal papilla situated at the base of the mainly epithelial hair follicle (see Fig. 2.2) [68, 92].

2.2.2 The Miniaturisation Processes

Scalp follicles pass through several cycles before the processes are complete (Fig. 2.2). Normally, scalp hair follicles are mainly in anagen; the average anagen of 2–3 years and telogen of approximately 100 days [73] gives an anagen-to-telogen ratio of about 9:1, although there is some seasonal variation in people living in temperate regions (see Sect. 2.3.1) [108]. While androgenetic alopecia develops, anagen shortens, increasing the proportion of telogen hairs [7, 97, 118, 140] which is detectable before any balding; it also results in shorter hairs. Follicle miniaturisation can be seen histologically [7, 74], indicating the hairs are also thinner [74, 118]. When scalp appears bald, most of the follicles are very short and small, with occasional resting terminal hairs.

Studies of androgenetic alopecia are complicated by senescent balding, the non-androgen-dependent hair thinning found in those more than 50 [28]. This also involves a progressive decrease in anagen follicles

[100] and hair diameter [28], but does not normally lead to baldness. Kligman suggested that both forms may occur together, proposing a pronounced inflammatory component in androgenetic alopecia, not seen in senescent baldness [74]. Recent observations have confirmed peri-follicular inflammation [30]. The sclerotic remains of the fibrous sheath are seen below the shortened follicles as "streamers" [74]; damage to the dermal sheath by chronic inflammation may prevent the reformation of terminal hair follicles in long-term alopecia, although this is debated.

During the miniaturisation processes of androgenetic alopecia, the follicle's associated arrector pili muscle reduces much more slowly than the follicle [82], while the androgen-dependent sebaceous gland becomes enlarged [74], often resulting in an oily, greasy scalp. Other changes include a reduced follicular blood supply [20, 111] and nerve networks twisting to form a type of encapsulated end organ below the follicle [41].

2.3 The Pathogenesis of Androgenetic Alopecia

Hair follicles are under hormonal regulation because of the importance of coordinating alterations in insulation properties and colour of an animal's coat to the environment [29, 107], and changes in the social and sexual communication aspects to the appropriate stage in the life cycle. In mammals, seasonal changes are coordinated to day length and, somewhat less, to temperature in the same way as seasonal breeding. Changes are translated to the follicle via the pineal and hypothalamus-pituitary route, involving gonadal, thyroid and corticosteroid hormones [29, 107].

2.3.1 Seasonal Changes in Human Hair Growth

Regular circannual changes in human hair growth were only fully recognised comparatively recently [19, 108]. In white Englishmen with indoor occupations, androgen-dependent beard and thigh hair growth increase significantly in the summer [108] and are lowest in January and February. This may reflect changes in circulating androgen levels, because these rise in European men in the summer [115, 125]. Scalp hair shows a single annual cycle with more than 90% of hairs growing in the spring, falling to about 80% in the autumn, paralleled by increased numbers of hairs being shed per day, which more than doubled [108]. Which hormones regulate this is unclear. As most people's scalp follicles will be in anagen for at least 2–3 years, such a marked seasonal effect is quite remarkable. Nevertheless, this effect has a major significance, as any new drug or treatment should be studied for at least a year to separate any effects from normal seasonal variations.

2.3.2 Paradoxical Effects of Androgens on Human Hair Growth

Androgens are the main regulator of human hair growth, although other hormones, including those of pregnancy, prolactin, melanocyte-stimulating hormone (MSH) and thyroid hormones, have effects in man and other species [105, 107]. One of the first signs of puberty is the gradual replacement of tiny vellus hairs with larger, more pigmented *intermediate* hairs in the pubis and later in the axillae [83, 84]; eventually, larger and darker terminal hairs are produced. These changes parallel the pubertal rise in plasma androgens that occurs earlier in girls than in boys [143, 144]. Similar changes occur in many areas in young men producing the beard, an extended pubic diamond, chest hair and greater hair on the limbs, which readily distinguish the mature adult man. These changes are gradual and often progress over many years. Beard growth increases rapidly during puberty, but continues to rise until the man is in his mid-30s [52], while terminal hair on the chest or ear canal may appear only years after puberty [50].

In marked contrast, androgens have no obvious effect on many follicles that produce terminal hairs in childhood, such as the eyelashes or many scalp follicles. Paradoxically, in individuals with a genetic pre-disposition, androgens promote the gradual transformation of large terminal scalp follicles to tiny vellus ones causing androgenetic alopecia [49, 51, 53]. Apart from the role of androgens, the precise mechanisms of these responses within the hair follicle are not well understood, although it is clear that the responses are intrinsic to the individual

follicle and dependent on body site. Not only do follicle responses range from stimulation to inhibition, but sensitivity to the androgens also varies within clearly defined patterns. Facial hair develops first above the mouth and centre of the chin in both young men and hirsute women, and regression in androgenetic alopecia occurs in a progressive manner, despite all follicles receiving the same circulating hormones [51]. Similarly, female circulating androgen levels are high enough to produce axillary and the female terminal pubic hair, but male patterns of body hair require normal male levels [4, 19, 49, 53, 58, 83, 84, 105, 115, 125, 143, 144]. Thus, androgens appear to promote and amplify an individual follicle's genetic programming. This end-organ response is the basis for hair transplant surgery [98]; when "non-balding" regions of the scalp are transplanted to the balding vertex, they retain their innate lack of androgen response and maintain terminal follicles, while miniaturisation progresses in the vertex follicles behind them.

2.3.3 Essential Requirement for Androgens

Androgens are essential for the development of androgenetic alopecia. It does not occur in men who have never entered puberty; men castrated after puberty show no further progression of their baldness, although they do not regain the frontal hairline, and testosterone replacements stimulate progressive balding, which halts during temporary withdrawal of the anagen [49, 52, 53].

Androgens, like other steroid hormones, pass through the plasma membrane and bind to specific intracellular proteins, inactive androgen receptors. This activates the receptors causing shape changes, which enable them to bind to specific hormone-responsive elements (HREs) in the DNA, often in association with other co-activating proteins, to initiate the translation of specific androgen-regulated genes and synthesis of their proteins (see Fig. 2.3, upper diagram).The essential role of androgens is confirmed by the absence of any post-pubertal changes in body or scalp hair growth in men without functional androgen receptors (i.e. with androgen insensitivity syndrome) [85]. Individuals with the complete form exhibit no pubic, axillary, chest or beard terminal hair and do not develop androgenetic alopecia.

Although testosterone is the main circulating androgen in men, in many tissues, it is metabolised intracellularly to the more potent androgen, 5α-dihydrotestosterone, by the enzyme 5α-reductase [17]. Both testosterone and 5α-dihydrotestosterone can activate the androgen receptor to alter the expression of androgen-sensitive genes. There are also various weaker androgens in the circulation, particularly in women, which can be metabolised to more active androgens such as testosterone and 5α-dihydrotestosterone (see Fig. 2.3). Deficiencies in 5α-reductase also reduce androgen effects on some hair follicles. Although all hair follicles require intracellular androgen receptors to respond to androgens, the necessity for 5α-reductase activity to produce intracellular 5α-dihydrotestosterone for the androgen response varies [54]. Individuals with 5α-reductase type 2 deficiency do not develop male patterns of body hair growth, despite their circulating androgens; they produce only female patterns of pubic and axillary hair, although their body shape masculinises [142]. They appear not to exhibit male pattern baldness, but this is more difficult to interpret; however, the re-growth of hair in young balding men given the 5α-reductase type 2 inhibitor, finasteride, strongly supports the role of both androgens and 5α-reductase in androgenetic alopecia [69].

Despite the widely held belief that baldness is an indicator of increased male sexuality, there is little scientific evidence for this other than the clear link with normal androgen parameters. There was no relationship between androgenetic alopecia and other androgen-regulated parameters, including muscle, bone, sebum excretion rate or body hair growth in adult men [9]. Normal male testosterone levels have been reported in balding men [101, 103] with higher urinary dehydroepiandrosterone [101] or dehydroepiandrosterone sulphate [113]; other studies showed raised serum-free testosterone, i.e. that are not bound to sex hormone-binding globulin [16, 26]. Overall, normal male androgen levels appear to be sufficient to produce androgenetic alopecia; the response obtained appears related to the intrinsic follicular response. In women, raised circulating androgens, particularly free androgens, appear to be related to hair loss, although the means from studies are often within the normal ranges for pre-menopausal women [8, 14, 25, 39, 79, 88, 129]. Women who present with androgenetic alopecia also often exhibit polycystic ovarian disease and hirsutism [14, 38, 91], even if presenting with alopecia without menstrual abnormalities [14]. Therefore, androgenetic alopecia requires circulating androgens, androgen receptors and intracellular 5α-reductase type 2.

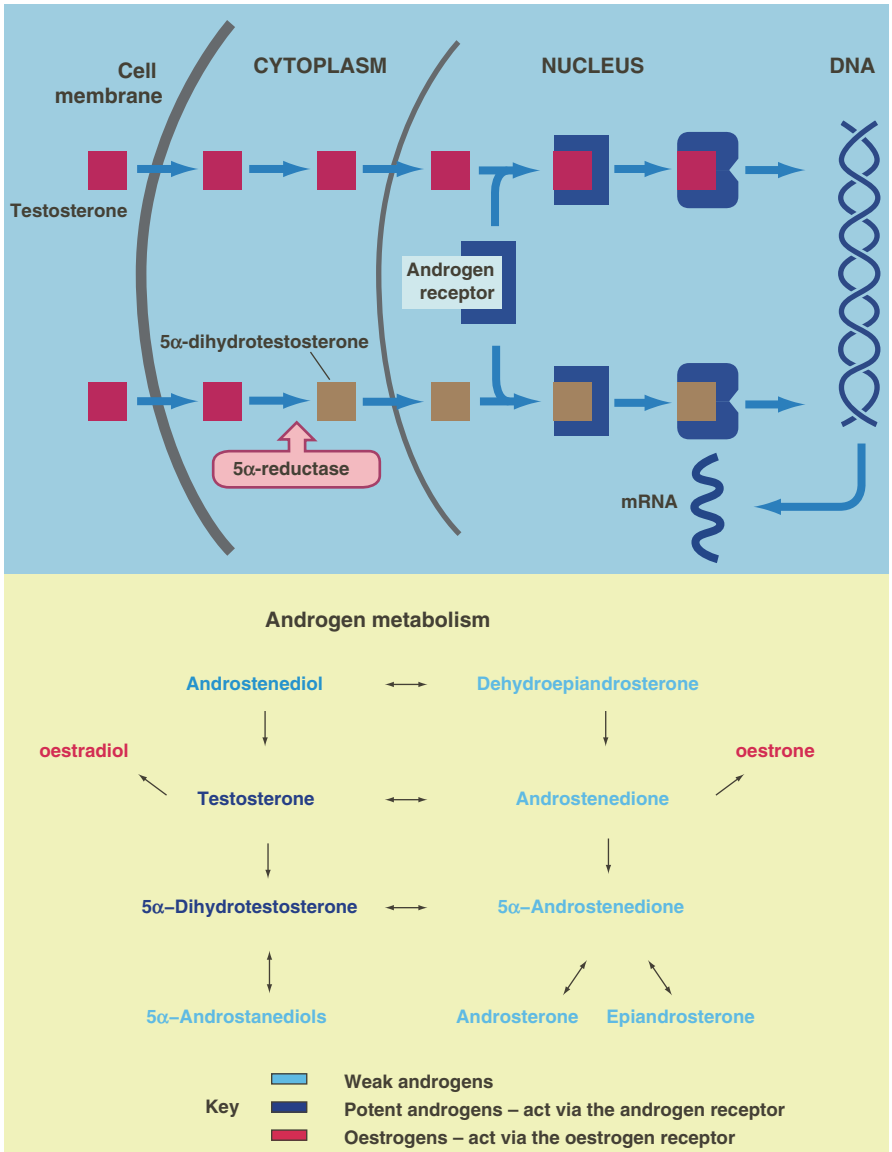

Fig. 2.3 The mechanism of action of androgens. *Upper diagram* – simple schematic of the general mechanism of androgen action. Androgens diffuse from the blood through the plasma membrane. Inside the cell, like other steroid hormones, testosterone may bind to specific androgen receptors. This occurs in many tissues such as skeletal muscle and axillary and pubic hair follicles. However, in certain tissues, particularly the secondary sexual organs such as prostate or beard and balding hair follicles, testosterone is metabolised to the more potent androgen, 5α-dihydrotestosterone (see *lower diagram*). If both are available in similar quantities, the receptor will bind 5α-dihydrotestosterone. Once hormone has bound, the receptor complex undergoes a conformational change exposing DNA-binding sites and the hormone-receptor complex, in conjunction with other co-activating proteins, will bind to specific hormone response elements (HREs) in the DNA altering the expression of specific androgen-dependent genes. *Lower diagram* – androgen metabolism. Circulating androgens such as testosterone from the testis in men and weaker androgens such as dehydroepiandrosterone and androstenedione from the adrenals and ovaries in women can be metabolised in many skin tissues. Some metabolism causes an increase in potency, e.g. from testosterone to 5α-dihydrotestosterone (DHT) as the androgen receptor binds DHT more strongly even than testosterone, another potent androgen. Other metabolisms form weaker androgens normally involved in excretion pathways, e.g. the androstanediols or steroids which act via the other steroid receptors i.e. the oestrogens

2.3.4 Genetic Influences

2.3.4.1 Incidence

The genetic involvement in androgenetic alopecia is also pronounced. It runs in families, and there are racial differences (see Sect. 2.1.2), while androgen replacement only stimulated balding in castrated men with a family history [49]. Although androgenetic alopecia has generally been accepted as an autosomal dominant trait with variable penetrance [5], this is based on a familial analysis in 1916 [99], and a more complex, polygenic inheritance is more likely [32, 76]. Interestingly, a very strong correlation in incidence was found in 54 sets of sons and fathers, with 81.5% of balding sons having balding fathers (Hamilton-Norwood scale III or higher) [32, 76]. This is greater than expected from an autosomal dominant inheritance and could implicate a paternally inherited gene, e.g. on the Y chromosome or the involvement of a gene that is capable of being paternally imprinted (i.e. preferentially inactivated by methylation of DNA, etc.).

2.3.4.2 Investigation of Specific Genes

Several genes have been investigated for association with androgenetic alopecia. No association was detected with neutral polymorphic markers for either type 1 (SRD5A1) or type 2 (SRD5A2) 5α-reductase genes in case–control association studies of Australian [32] or Korean (Asian) men [45]. A later study did find an association with a mutant allele (A49T) of type 2 5α-reductase, but this decreased the incidence of alopecia, although increasing that of prostate cancer [55]! Known dimorphic and polymorphic markers within the androgen receptor gene are more linked to balding in Caucasian men [34]. The *Stu* I restriction fragment length polymorphism (RFLP) in exon 1 was present in 98% of 54 young balding men and 92% of 392 older balding men but was also found in 77% of their older, non-balding controls. Analysis of triplet repeat polymorphisms, CAG and GAC revealed significantly higher incidence of short/short polymorphic CAG/GGC haplotypes in balding subjects and lower short/long, although no significance was provided. Interestingly, shorter triplet repeat lengths are associated with precocious puberty, i.e. appearance of pubic hair before eight [63] and androgen-dependent prostate cancer [127]. Whether this has functional significance, such as increased androgen sensitivity, or simply reflects linkage disequilibrium with a causative mutation is not clear. However, when the binding capacity for a range of steroids was compared between androgen receptors from balding and non-balding follicle dermal papilla cells, no differences were detected [58], and no link was seen with increased copy number variations of the androgen receptor gene [17].

Recently, genetic variability in a 1 Mb region within and centromeric to the androgen receptor gene was found to be associated with androgenetic alopecia [60] and strongest risk when associated with a variant in the flanking ectodysplasin A2 receptor gene (EDA2R) [61]. Links with a locus on chromosome 20 (20 pll) have also been reported in several populations [60, 117]. Other genes have also been implicated, including a link to one allele of the steroid metabolism gene, CYP17, to both women with polycystic ovaries and their brothers with early onset androgenetic alopecia [11]. An interesting connection is severe, early onset androgenetic alopecia in men with the x-linked gene for adrenoleukodystrophy who tend to have low testosterone levels [75]. The gene for *hairless*, which results in a complete loss of hair [1], also showed a marginally significant correlation with androgenetic alopecia with two mutations, but these became insignificant after correction for multiple testing [59]. The situation is still not fully clear at the moment, but moving forward rapidly.

2.4 Current Model for Androgen Action in the Hair Follicle

The mesenchyme-derived dermal papilla plays an important regulatory role in the follicle, determining the type of hair produced [68, 92]. Since hair follicles appear to partially recapitulate embryogenesis during the hair cycle (Fig. 2.2, Sect. 2.2.1) and steroids act via the mesenchyme in many developing steroid-dependent tissues [22], the author proposed that androgens would act on the other components of the follicle via the dermal papilla [105, 109]. In this hypothesis (Fig. 2.4), circulating androgens enter the dermal papilla via its own blood capillaries, binding to androgen receptors within the dermal papilla cells of androgen-dependent hair follicles [108, 106, 109]. Whether or not they are first metabolised intracellularly to 5α-dihydrotestosterone depends on the site of the follicle;

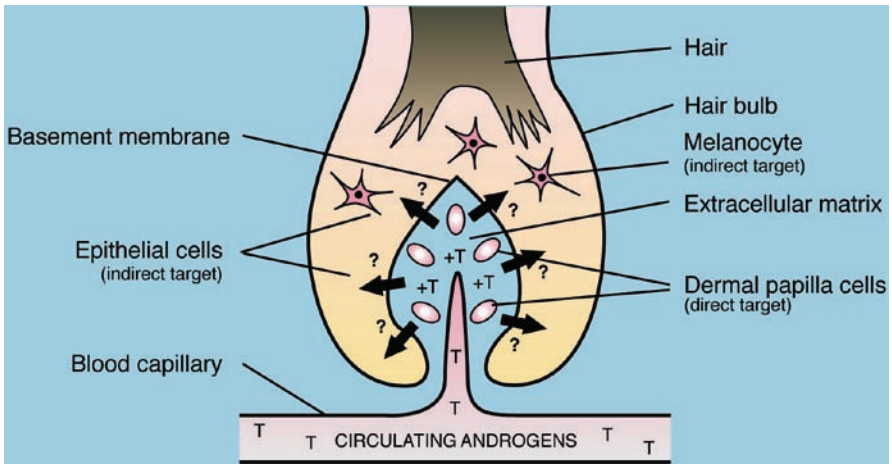

Fig. 2.4 Model of androgen action in the hair follicle. In the current hypothesis, androgens from the blood enter the hair follicle via the dermal papilla's blood supply. If appropriate, they are metabolised to 5α-dihydrotestosterone (see Fig. 2.3). They bind to androgen receptors in the dermal papilla cells causing changes in their production of regulatory paracrine factors; these then alter the activity of dermal papilla cells, follicular keratinocytes and melanocytes. *T* testosterone; *question mark* unknown paracrine factors. Reproduced from Randall [132]

for example, beard and balding-scalp follicle cells would first metabolise testosterone with 5α-reductase type 2, but axillary and pubic cells would not (Fig. 2.3). After androgens bind their receptors, the gene expression of the dermal papilla cells is altered so that they change their production of regulatory factors such as soluble growth factors or extracellular matrix proteins [105, 109]. Targets include the keratinocytes and melanocytes (pigment producing cells) and also the cells of the follicular connective tissue sheath, the dermal vasculature, and even dermal papilla cells themselves because all these would be altered in the formation of a differently sized or coloured hair; beard and scalp dermal papilla cells do produce autocrine stimulatory factors [47, 133]. Therefore, the direct androgen target cells would be the dermal papilla cells, and the other follicular components would be indirectly controlled by androgens. This seems a realistic model because androgens have such widely differing effects on follicles in different body sites, including whether or not 5α-reductase type 2 is necessary for stimulation of hair growth. It would be difficult for the follicle to be so well controlled if each follicular target cell had to respond to androgens directly.

This hypothesis has now received much experimental support. Androgen receptors are localised to the dermal papilla, but not keratinocyte cells by immunohistochemistry [15, 67] and cultured dermal papilla cells derived from androgen-sensitive beard and balding scalp follicles contain specific, saturable androgen receptors [2, 58, 110]. Important corroboration also comes from studies of androgen metabolism by cultured dermal papilla cells, because this reflects hair growth in 5α-reductase deficiency; beard cells produce 5α-dihydrotestosterone and express genes 5α-reductase type 2 [2, 65, 132] but pubic cells do not [48], corresponding to the presence of pubic but not beard hair in patients.

Although the key role of the dermal papilla in the induction of hair follicles and the regulation of the type of hair produced is well established [68], the lower part of the connective tissue sheath, or dermal sheath, which surrounds the hair follicle and isolates it from the dermis, has also been shown to form a new dermal papilla and human hair follicle development [116]. Cultured beard dermal sheath cells contain similar levels of androgen receptors to beard dermal papilla cells [86], and balding scalp dermal sheath expresses the mRNA for 5α-reductase type 2 like the dermal papilla [3]. Dermal sheath cells may respond directly to androgens to facilitate change in size of the sheath, or even the dermal papilla, in the development of a new anagen follicle; this would enable the new follicle to be larger or smaller depending on the follicle's androgen response. These results merit a modification of the model to include a direct action of androgens on the lower dermal sheath too.

Since the production of paracrine regulators by the dermal papilla is so important for normal follicle functions and androgen regulation (Fig. 2.4), it has been

investigated by several groups. Cultured dermal papilla cells retain hair growth-promoting ability [68] and secrete both extracellular matrix factors and soluble, proteinaceous growth factors [109]. Bioassays demonstrate that human cultured dermal papilla cells can secrete soluble, proteinaceous factors that promote growth in other dermal papilla cells [109, 132], outer root sheath cells [66] and transformed epidermal keratinocytes [56]. Importantly, testosterone in vitro alters the mitogenic capacity in line with its effect on hair growth in vivo. Testosterone stimulated beard, but not scalp, cells to increase their growth-promoting effects for beard dermal papilla cells [132], outer root sheath cells [66] and keratinocytes [56], while decreasing the capability of androgenetic alopecia scalp dermal papilla cells both from men [56] and the stump-tailed macaque [90]. Research is currently focussed on identifying androgen-regulated factors (reviewed [111]); androgens in vitro increase IGF-1 production by androgen-dependent beard cells [66] while stem cell factor (SCF) is produced in higher amounts by beard cells than control, non-balding scalp cells [57] and less by balding cells [114], presumably in response to androgens in vivo. Because SCF is the ligand for the cell surface receptor, c-kit, found on human follicular melanocytes, this may play a role in androgen-potentiated changes in hair pigmentation. In androgenetic alopecia where the hairs are paler than normal scalp hairs, the concentration of melanocytes per unit area of the hair bulb is the same in the paler, intermediate hair follicles of balding areas as in normal scalp follicles and they retain the same levels of the c-kit receptor protein. The only difference detected was the reduced SCF production by balding dermal papilla cells [114].

The expression of mRNA for the protease nexin-1 in dermal papilla cells is also altered by androgens [126]. This may play a role by altering the amount of extracellular matrix components produced [112] and therefore the size of the follicle and hair [6, 31]. Recently, dermal papilla cell conditioned media from balding scalp follicles has been shown to inhibit the growth of both human and rodent whisker dermal papilla cells in vitro and delay mouse hair growth in vivo [46], suggesting the active secretion of an inhibitory factor or factors. A possible candidate is transforming growth factor-β1 (TGF-β1), which has been induced by androgens in balding dermal papilla cells with transfected androgen receptors [64]. TGF-β also inhibits hair follicle growth in vitro [102] and a probable suppressor of TGF-β1 delayed catagen progression in mice in vivo [56]. Other candidates include dickkofp-1 (DDK-1) [87] and Wnt signalling [72] which are both induced by 5α-dihydrotestosterone in balding dermal papilla cells. Further study of such factors should lead to better treatments for androgen-dependent hair follicle disorders.

2.5 Treatment

Mainly because the pathogenesis mechanisms of androgenetic alopecia are not fully understood, the treatments available are limited and vary in effectiveness. Over the centuries, a wide range of remedies have been suggested for androgenetic alopecia [78] and currently treatments include wigs and hairpieces, surgery, hormone action modifiers, and non-hormonal therapy. Several of these are based on our understanding of the mechanisms of androgen action within the follicle.

2.5.1 Surgery

All surgical methods capitalise on the different intrinsic responses to androgens by spreading “non-balding”, i.e. occipital and parietal, terminal follicles over the androgen-sensitive scalp regions [98]. Originally involving the transplant of small biopsies with several follicles, this usually now involves micro-grafts with one or two follicles [134]. Once established, these expensive and painful treatments are long-lasting; however, the effect can be marred by the continual natural progression of balding, which may well require further transplants to avoid isolation of the transplanted region. Future modifications may include culturing dermal papilla cells to expand the non-balding follicular material before replanting into balding regions.

2.5.2 Hormonal Treatments

2.5.2.1 Anti-Androgens

Blocking the activation of androgen receptors by anti-androgens is a theoretically useful approach, but not really practical because anti-androgens block all

androgen actions, with unacceptable side effects on male masculinity and the potential to cause feminisation of a male foetus in a pregnant woman. Nevertheless, cyproterone acetate, an anti-androgen with pro-gestational effects, established for hirsutism and acne in Europe and Canada [37], is also used for androgenetic alopecia in women, generally in combination with oestrogen as oral contraception in pre-menopausal women; treatment appears to stabilise progression [138]. Similarly, spironolactone, an aldosterone antagonist with mild anti-androgenic effects, is often used in the USA [62].

2.5.2.2.5 α-Reductase Inhibitors

The most successful current therapeutic treatment in men is oral finasteride, a 5α-reductase type 2 inhibitor, which blocks the conversion of testosterone to 5α-dihydrotestosterone [122]. Finasteride, developed for benign prostate hypertrophy [141], slows hair loss progression and can promote hair growth in young men with below stage V hair loss (Fig. 2.1); it can also be useful in older men [44, 69–71]. Whether the inhibitor is working centrally or within the balding follicles is unclear because plasma 5α-dihydrotestosterone levels are reduced [69]. Unfortunately, finasteride was not effective in post-menopausal women [104], and its use in pre-menopausal women is restricted like anti-androgens (Sect. 2.5.2.1). Recently, a short trial of dutasteride, a dual inhibitor of 5α-reductase types 1 and 2 has shown similar, possibly better, effects [96].

2.5.2.3 Non-Hormonal Therapy

The most commonly used non-hormonal treatment, minoxidil, was initially devised as a vasodilator for use as an anti-hypertensive drug, but stimulated excessive hair growth as a side effect [23, 87, 123]. This provoked major interest in hair follicle biology because vellus follicles were stimulated to form terminal hairs, previously believed impossible; a reversal of the normal scheme where greater understanding leads to new approaches! Topical application of minoxidil is used in both men and women [23, 87, 123], stimulating re-growth in up to 30%, with only about 10% obtaining complete re-growth [95, 120]. Most success occurs with younger men and with the early stages of balding. Minoxidil probably acts as a potassium channel regulator of ATP-sensitive potassium channels [124].

2.5.3 Future Developments

Androgenetic alopecia is a common, progressive, androgen-dependent hair disorder with strong genetic links that often has marked negative effects on the quality of life. There is a great deal of interest among pharmaceutical companies ever since minoxidil demonstrated that terminal hair growth could be re-stimulated from balding follicles. The development of the 5α-reductase type 2 inhibitor, finasteride, has opened up the use of hormonal treatments in men. However, as the hormonal trigger and the follicle's ability to respond persist, treatment must be maintained.

Take Home Pearls

- Over the last 20 years, there have been great improvements in our understanding of hair follicle biology and the mechanism of androgen action in hair follicles, establishing the importance of local biochemical and cellular interactions within the follicle. This has highlighted the significant role of the dermal papilla, particularly in androgen regulation.
- Recently, dermal papilla cells have been shown to secrete autocrine and paracrine factors, which may play a role in the pathogenesis of androgenetic alopecia. These are currently a focus of investigation; IGF-1, SCF and TGF-β are implicated in androgen action.
- The significant role of inherited genetic predisposition to androgenetic alopecia is also being clarified, revealing strong associations with aspects of the androgen receptor gene and others on chromosome 20.
- Understanding the molecular mechanisms has led to treatments such as finasteride; as our knowledge deepens further, novel therapies should be developed.

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Inflammatory Phenomena and Fibrosis in Androgenetic Alopecia

3

Ralph M. Trüeb

Core Message

› In androgenetic alopecia the genetically predisposed hair follicles are susceptible to androgen-stimulated hair follicle miniaturization, leading to replacement of large and pigmented hairs by barely visible depigmented hairs. The result is a progressive decline in visible scalp hair density that follows a defined, age- and sex-dependent pattern. Major advances have been achieved in understanding peculiarities of the androgen metabolism involved. Nevertheless, clinical practice has shown that simply blocking androgens has only limited success. On histologic examination, the miniaturization of terminal hairs is frequently associated with perifollicular inflammatory phenomena, and eventually fibrosis. Therefore, sustained microscopic follicular inflammation with connective tissue remodeling, eventually resulting in permanent hair loss, is considered a possible cofactor in the complex etiology of androgenetic alopecia.

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3.1 Pathobiology of Androgenetic Alopecia and Current Pharmacologic Treatment

Androgenetic alopecia, which is also referred to as male-pattern hair loss or common baldness in men and as female-pattern hair loss in women, affects at least 50% of men by the age of 50 years, and up to 70% of all males in later life [17]. Estimates of its prevalence in women have varied widely, though recent studies claim that 6% of women aged less than 50 years are affected, increasing to a proportion of 30–40% of women aged 70 years and over [18].

The hair loss is heritable, androgen-dependent, and occurs in defined age- and sex-dependent patterns. While male pattern AGA is characterized by its typical bitemporal recession of hair and balding vertex, female pattern AGA is set apart by its diffuse thinning of the crown and intact frontal hairline.

It is assumed that the genetically predisposed hair follicles are the target for androgen-dependent, gradual replacement of large and pigmented terminal hairs by barely visible depigmented vellus hairs in affected areas [20]. The result is a progressive decline in visible scalp hair density. The process is characterized by progressive shortening of the duration of the growing or anagen phase of the hair cycle with successive cycles, leading to decreased numbers of hair in anagen at any given time, and progressive follicular miniaturization with conversion of terminal to vellus-like follicles [20]. The result is increased shedding of short-lived hairs in the resting or telogen phase of the hair cycle (telogen effluvium), while the affected hair follicles produce shorter, finer hairs that cover the scalp poorly. Since androgenetic

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alopecia involves processes of premature termination of anagen associated with premature entry into catagen, it is critically important to dissect the molecular controls of the anagen-catagen transformation of the hair cycle [19]: Catagen or the transition phase of the hair cycle has been suggested to occur as a consequence of decreased expression of anagen maintaining factors, such as insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), and increased expression of cytokines promoting apoptosis, such as transforming growth factor beta 1 (TGFβ 1), interleukin-1alpha (IL-1α), and tumor necrosis factor alpha (TNFα).

While the genetic involvement is pronounced but poorly understood, major advances have been achieved in understanding principal elements of the androgen metabolism involved [7]: Androgen-dependent processes are predominantly due to the binding of dihydrotestosterone (DHT) to the androgen receptor (AR). DHT-dependent cell functions depend on the availability of weak androgens, their conversion to more potent androgens via the action of 5alpha-reductase, low enzymatic activity of androgen inactivating enzymes, and functionally active AR present in high numbers. The predisposed scalp exhibits high levels of DHT, and increased expression of the AR. Conversion of testosterone to DHT within the dermal papilla plays a central role, while androgen-regulated factors deriving from dermal papilla cells are believed to influence growth of other components of the hair follicle. Responses to androgens are obviously also intrinsic to the individual hair follicle: Not only does the response vary from stimulation to inhibition of hair growth depending on the body site, but androgen sensitivity also varies within individual areas, i.e., regression in AGA occurs in a patterned, progressive manner. Since many extrinsic hair growth-modulatory factors, such as androgens [22], apparently operate at least in part via the dermal papilla, research is currently also focused on identifying androgen-regulated factors deriving from dermal papilla cells. Of the several factors that have been suggested to play a role in hair growth, so far only IGF-1 has been reported as altered in vitro by androgens [5], and stem cell factor (SCF) has been found to be produced in higher amounts by androgen-dependent beard cells than in control nonbalding scalp cells, presumably also in response to androgens [4].

The aim of therapy is to increase hair coverage of the scalp and to retard progression of hair thinning. Current available treatment modalities with proven efficacy are oral finasteride, a competitive inhibitor of type 2 5alpha-reductase, and topical minoxidil, an adenosine-triphosphate-sensitive potassium channel opener which has been reported to stimulate the production of VEGF in cultured dermal papilla cells.

The rationale for the use of finasteride to treat AGA in men is based on the absence of androgenetic alopecia in men with congenital deficiency of type 2 5alpha-reductase, and the presence of increased 5alpha-reductase activity and DHT levels in balding scalp [8].

Minoxidil promotes hair growth through increasing the duration of anagen. It causes hair follicles at rest to grow, and enlarges suboptimal follicles. While minoxidil was developed for treatment of hypertension, and this feature of the drug's action is best understood, its mechanism of action on hair growth is poorly understood. Minoxidil is a potassium-channel opener and vasodilator, and has been reported to stimulate the production of VEGF in cultured dermal papilla cells [12]. There is evidence that this effect is mediated by adenosine and sulfonylurea receptors, which are well-known target receptors for adenosine-triphosphate-sensitive potassium channel openers [13].

The limited success rate of treatment of androgenetic alopecia with finasteride and minoxidil means that further pathogenic pathways may be taken into account. On histologic examination of scalp biopsies, the miniaturization of terminal hairs is frequently associated with perifollicular lymphocytic infiltration, and eventually fibrosis [6, 29]. Therefore, it is conceivable that the role of this microscopic follicular inflammation causing fibrosis below the shortened balding follicle has been underestimated, though it seems likely that this would prevent the follicle to reform a terminal hair follicle.

3.2 Hair Follicle Microinflammation and Fibrosis

The implication of microscopic follicular inflammation in the pathogenesis of androgenetic alopecia has emerged from several independent studies [6, 15, 29]: An early study referred to an inflammatory infiltrate of activated T cells and macrophages in the upper third of the hair follicles, associated with an enlargement of the follicular dermal sheath composed of collagen bundles (perifollicular

fibrosis) in regions of actively progressing alopecia [6]. Horizontal section studies of scalp biopsies indicated that the perifollicular fibrosis is generally mild, consisting of loose, concentric layers of collagen that must be distinguished from cicatricial alopecia [29]. The term "microinflammation" has been proposed, because the process involves a slow, subtle, and indolent course, in contrast to the inflammatory and destructive process in the classical inflammatory scarring alopecias [15].

The significance of these findings has remained controversial. However, morphometric studies in patients with male-pattern androgenetic alopecia treated with minoxidil showed that 55% of those with microinflammation had regrowth in response to treatment, in comparison to 77% in those patients without inflammation and fibrosis [29].

An important question is how the inflammatory reaction pattern is generated around the individual hair follicle. Inflammation is regarded as a multistep process, which may start from a primary event. The observation of a perifollicular infiltrate in the upper follicle near the infundibulum suggests that the primary causal event for the triggering of inflammation might occur near the infundibulum [15]. On the basis of this localization and the microbial colonization of the follicular infundibulum with *Propionibacterium spp*., *Staphylococcus spp.*, *Malassezia spp.*, or other members of the transient flora, one could speculate that microbial toxins or antigens could be involved in the generation of the inflammatory response. The production of porphyrins by *Propionibacterium spp.* in the pilosebaceous duct has also been considered to be a possible cofactor of this initial pro-inflammatory stress [15].

Alternatively, keratinocytes themselves may respond to chemical stress from irritants, pollutants, and UV irradiation, by producing radical oxygen species and nitric oxide, and by releasing intracellularly stored IL1-α. This pro-inflammatory cytokine by itself has been shown to inhibit the growth of isolated hair follicles in culture [21]. Moreover, adjacent keratinocytes, which express receptors for IL-1, start to engage the transcription of IL-1 responsive genes: mRNA coding for IL-1β, TNFα, and IL-1α, and for specific chemokine genes, such as IL-8, and monocyte chemoattractant protein-1 (MCP-1) and MCP-3, themselves mediators for the recruitment of neutrophils and macrophages, have been shown to be upregulated in the epithelial compartment of the human hair follicle [15]. Besides, adjacent fibroblasts are also fully equipped to respond to such a pro-inflammatory signal. The upregulation of adhesion molecules for blood-borne cells in the capillary endothelia, together with the chemokine gradient, drive the transendothelial migration of inflammatory cells, which include neutrophils through the action of IL-8, T cells and Langerhans cells at least in part through the action of MCP-1. After processing of localized antigen, Langerhans cells, or alternatively keratinocytes, which may also have antigen presenting capabilities, could then present antigen to newly infiltrating T lymphocytes and induce T-cell proliferation. The antigens are selectively destroyed by infiltrating macrophages, or natural killer cells.

On the occasion that the causal agents persist, sustained inflammation is the result, together with connective tissue remodeling, where collagenases, such as matrix metalloproteinase (also transcriptionally driven by pro-inflammatory cytokines) play an active role [15]. Collagenases are suspected to contribute to the tissue changes in perifollicular fibrosis.

3.3 Red Scalp

Red scalp was first described by Thestrup-Pedersen and Hjorth [24], and subsequently commented on by Moschella [16], who stated on the difficult problem of "diffuse red scalp disease which can also be itchy and burning. … It is non responsive to any therapy including potent topical steroids or anti-seborrheic therapy." Patients frequently report aggravation in the sun, or report episodes of sunburn of the scalp (personal observation).

Grimalt et al. [3] presented their findings in 18 patients with "red scalp syndrome" at the 2000 Annual Meeting of the European Hair Research Society: The majority was middle-aged females consulting for hair loss. By definition no specific dermatologic disease was found. The scalp redness was associated with androgenetic alopecia in 13 out of 18 patients, and three of 10 biopsies performed were compatible with a cicatricial alopecia (not otherwise specified). Some patients reported associated discomfort of the scalp. The term *trichodynia* was first proposed for discomfort, pain, or paresthesia of the scalp related to the complaint of hair loss [23]. Subsequently this was found to be a frequent phenomenon [2], though its cause remains obscure. The most prevalent speculations with respect to its pathogenesis are perifollicular

inflammation and increased expression of the neuropeptide substance P in the vicinity of affected hair follicles [25]. In our published series of 403 patients complaining of hair loss examined for trichodynia, the dermatoscopic finding of scalp telangiectasia was found to strongly correlate with presence of trichodynia [30] (Figs. 3.1 and 3.2). An interesting analogy exists to rosacea, where patients with the telangiectatic variant of rosacea reported stinging sensation to the topical application of 5% lactic acid on the cheeks more frequently than patients with the papulopustular type of rosacea or normal controls [14]. On the one hand, these findings suggest a connection between sensory or subjective irritation and cutaneous vascular reactivity. On the other hand, dilated and tortuous vessels are typically found in photodamaged skin [9].

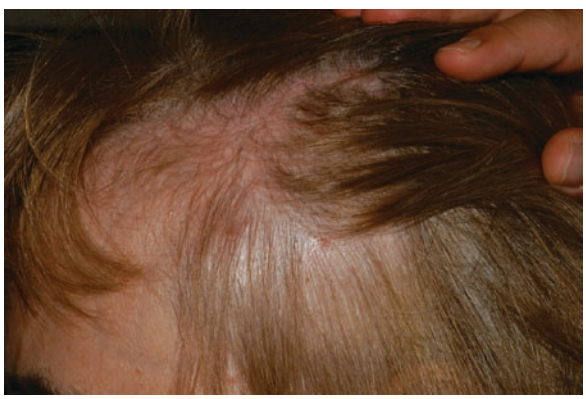


Fig. 3.1 Red scalp

Fig. 3.2 Red scalp. Dermoscopic finding of teleangiectasia. Note singular follicular pustule in the center

3.4 Fibrosing Alopecia in a Pattern Distribution

Alopecia in a pattern distribution is a common event associated with androgenetic hair loss and aging. Although it is regarded as a pathologic process by some physicians and many affected patients, by others it is considered a genetically determined physiologic event in the lives of most men and women. The same controversy applies to the histological finding of inflammatory cells in the vicinity of the upper hair follicle in androgenetic alopecia inasmuch as it remains uncertain whether this phenomenon is still a physiologic event or already reflects a pathologic process. Clinically, AGA is usually a noninflammatory and nonscarring process that eventually leads to permanent hair loss of the affected scalp.

In the recently described fibrosing alopecia in a pattern distribution [31], patients display progressive scarring alopecia in a pattern distribution (Fig. 3.3). Close clinical examination reveals obliteration of follicular orifices, perifollicular erythema (Fig. 3.4), and follicular keratosis, limited to the area of androgenetic hair loss (Fig. 3.5). Histological findings of androgenetic alopecia, i.e., increased numbers of miniaturized

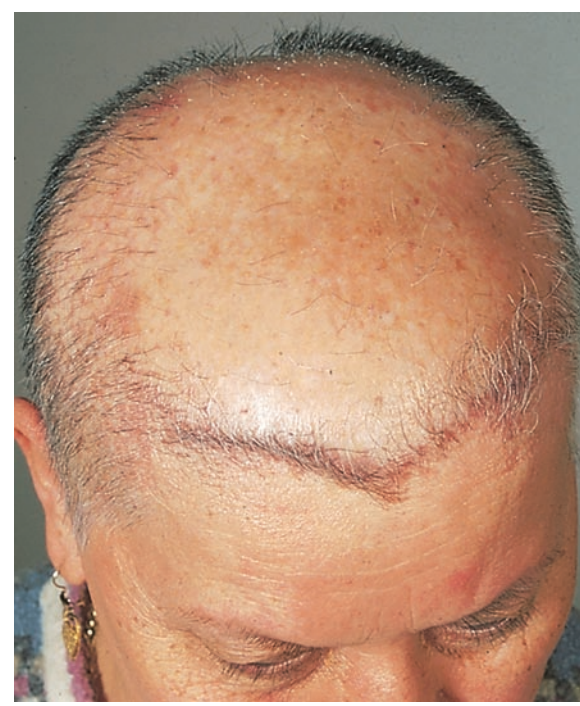


Fig. 3.3 Fibrosing alopecia in a pattern distribution (from Trüeb [27])

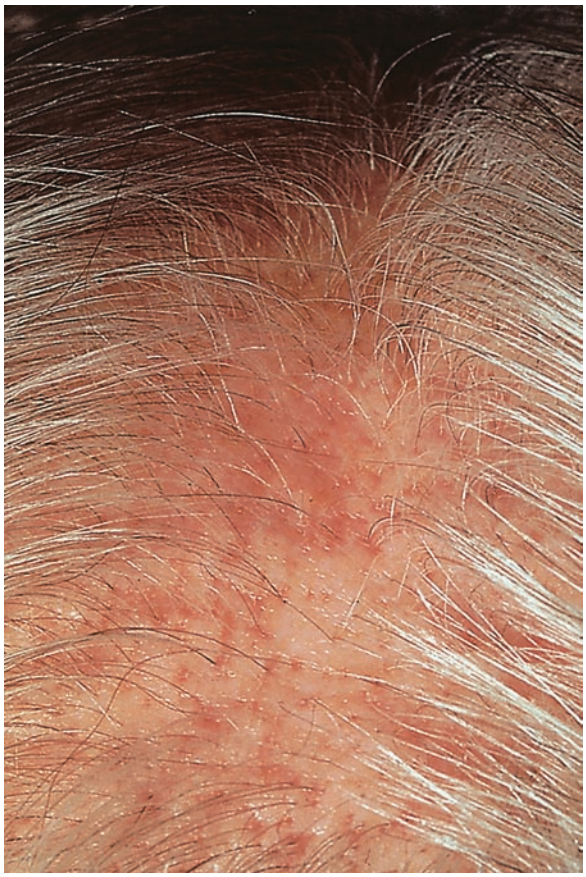


Fig. 3.4 Fibrosing alopecia in a pattern distribution (detail): Loss of follicular orifices and perifollicular erythema (from Trüeb [27])

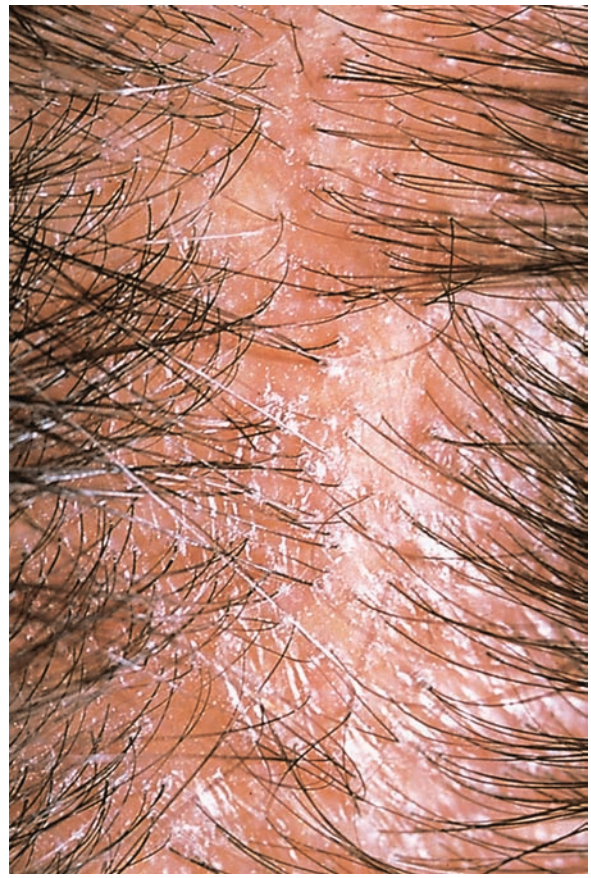


Fig. 3.5 Fibrosing alopecia in a pattern distribution (detail): Loss of follicular orifices and follicular keratosis (from Trüeb [27])

hair follicles with underlying fibrous streamers, are evident in the majority of patients and associated with a perifollicular lymphocytic infiltrate. A pattern of follicular interface dermatitis targeting the upper follicle is found in early lesions, whereas perifollicular lamellar fibrosis and the presence of selectively fibrosed follicular tracts characterize late lesions.

The pattern distribution and histological findings share features with progressive frontal fibrosing alopecia observed in postmenopausal women (Fig. 3.6) [31]. The clinical presentation in these women might mimic male pattern alopecia because it produces frontal recession of the hairline, but it is associated with clinical evidence of scarring. Kossard et al. [10] proposed the term postmenopausal frontal fibrosing alopecia for this presentation and later interpreted this type of alopecia as a frontal variant of lichen planopilaris on the basis of histopathologic and immunohistochemical studies [11]. Considerable overlap exists among postmenopausal frontal fibrosing alopecia, lichen planopilaris, and fibrosing alopecia in a pattern distribution: postmenopausal frontal fibrosing alopecia has been described in association with lichen

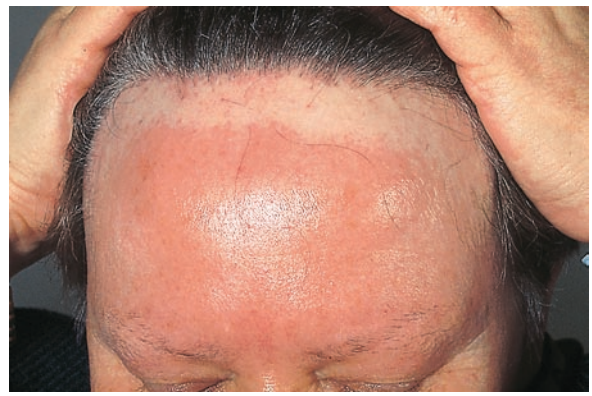
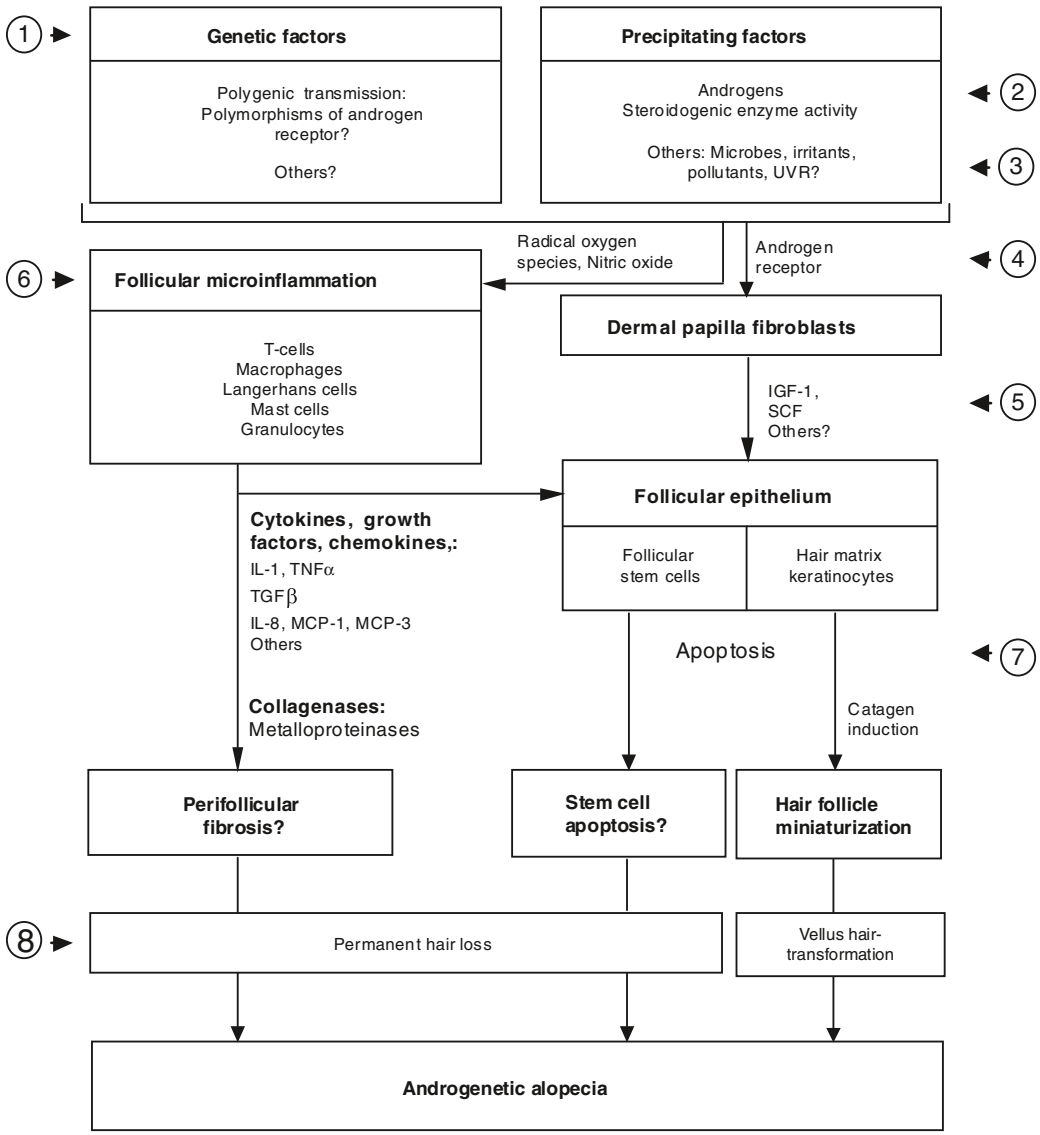


Fig. 3.6 Postmenopausal frontal fibrosing alopecia (from Trüeb [27])



Therapeutic strategies:

1. Gene therapy? (currently not available)
2. Modifiers of androgen metabolism: finasteride (available for men)
3. Antimicrobial shampoos?
4. Antiandrogens: cyproterone acetate (available for women)
5. Hair growth promoters: minoxidil (available for men and for women)
6. Antiinflammatory agents?
7. Apoptosis modulating agents? (currently not available)
8. Hair transplantation (available), implantation of dermal papilla cells or cells of follicle dermal-sheath (impending)

Diagram 3.1 Pathogenic mechanisms of androgenetic alopecia and therapeutic strategies (from Trüeb [26])

planus elsewhere (oral cavity) [28], and Zinkernagel and Trüeb [31] observed postmenopausal frontal fibrosing alopecia-type changes in patients with fibrosing alopecia in a pattern distribution.

Remarkably, in healthy murine skin clusters of perifollicular macrophages have been described as perhaps indicating the existence of a physiological program of immunologically controlled hair follicle degeneration by which malfunctioning follicles are removed by programmed organ deletion [1]. Various forms of clinically perceptible, permanent alopecia might represent pathological exaggeration of this type of programmed organ deletion, resulting in a lichenoid tissue reaction pattern and true scarring alopecia. Further studies are required in patients with fibrosing alopecia in a pattern distribution to elucidate a presumable role of androgenetic factors in addition to that of the lymphohistiocytic infiltrate, perifollicular lamellar fibrosis, and apoptosis-mediated follicular regression. An important question to be addressed in further studies is how the lichenoid tissue reaction pattern is generated around the individual androgenetic hair follicle. Follicles with some form of damage or malfunction might express cytokine profiles that attract inflammatory cells to assist in damage repair or in the initiation of apoptosis-mediated organ deletion. Alternatively, an as yet unknown antigenic stimulus from the damaged or malfunctioning hair follicle might initiate a lichenoid tissue reaction in the immunogenetically susceptible individual. The possible role of microbial antigens or superantigens in this context remains to be elucidated.

3.5 Targeting the Inflammatory Component in Androgenetic Alopecia

Clinical and investigative advances have helped us to understand some of the pathogenic steps leading to androgenetic hair loss (Diagram 3.1). Besides androgens and genetic imbalance, additional pathogenic factors are suspected, such as microbial flora, endogenous and exogenous stress, microinflammation, and possibly others. While further suspects are likely to be exposed, individual diversity of causal agents, as well as of the sequence of events or combined factors, must be kept in mind when addressing the biological conditions contributing to androgenetic alopecia.

So far, the inflammatory component has not been included in treatment protocols for androgenetic alopecia. Dissecting the molecular controls of immune-mediated, physiological hair follicle degeneration by apoptosis-mediated organ deletion could provide insights into how progression of some forms of permanent alopecia might be halted, which can be suppressed with only limited success by current treatment modalities. This could also hold true for further studies in androgenetic alopecia with inflammatory phenomena and fibrosis.

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Post-Menopausal Frontal Fibrosing Alopecia

4

Steven Kossard

Core Messages

- Post-menopausal frontal fibrosing alopecia is a distinctive form of alopecia associated with progressive hairline recession that is permanent and seen particularly in elderly women.
- Skin biopsies of the receding hairline reveal a lymphocyte-mediated follicular destruction identical to that seen with lichen planopilaris.
- This unusual alopecia may hold the key to understanding the complex relationship of pattern alopecia, sex-related differences in pattern alopecia and the triggers for autoimmune follicular destruction that may be unveiled with senescence.

4.1 Background

Post-menopausal frontal fibrosing alopecia (PFFA) is a distinct form of alopecia that was initially described in 1994 [10]. Subsequently, this unusual alopecia has been recognised in many dermatological centres worldwide with over 100 cases reported [2, 3, 4, 7, 8, 12, 16, 17, 18, 22, 24]. The striking symmetrical frontal and often temporoparietal hairline recession with permanent loss of hair follicles occurs predominantly in elderly post-menopausal women. This entity is of particular interest in reference to ageing hair as it appears to be an age-related problem. The condition provides a particular challenge, as it is not a universal phenomenon and the basis for this particular pattern of hair loss remains unknown. The hair loss is heralded by perifollicular erythema at the anterior hairline and progresses as a symmetrical recession that does not strictly follow the pattern of hair loss seen with androgenetic alopecia. The main pathology is a lymphocyte-mediated follicular destruction, which belongs to the spectrum currently classified as lichen planopilaris [15], an autoimmune disorder specifically targeting follicles.

4.2 Clinical Features

PFFA is seen almost exclusively in elderly women, often years after the menopause. This pattern of alopecia has been seen occasionally in premenopausal women [1, 6, 9, 12, 16, 24] and rarely in men [11, 21, 26] in whom the term frontal fibrosing alopecia (FFA) is more appropriate. The hair loss may be sudden and results in rapid recession of the frontal hairline and loss of eyebrows followed by a slow progressive course. In other women, the hair loss is slowly progressive and initially subtle. The follicles at the receding hairline usually have perifollicular erythema and slight perifollicular scale as the evidence of active follicular inflammation. The symmetric and progressive hairline recession produces a distinct pattern that can readily be recognised (Fig. 4.1). The skin in the area of total hair loss is often pale and smooth without visible follicular orifices and in some women, there a distinct contrast with adjacent aged sun-damaged skin. The extent of recession can be measured to monitor progression. In 50–80% of women, partial or complete loss of eyebrows is observed and may be the initial

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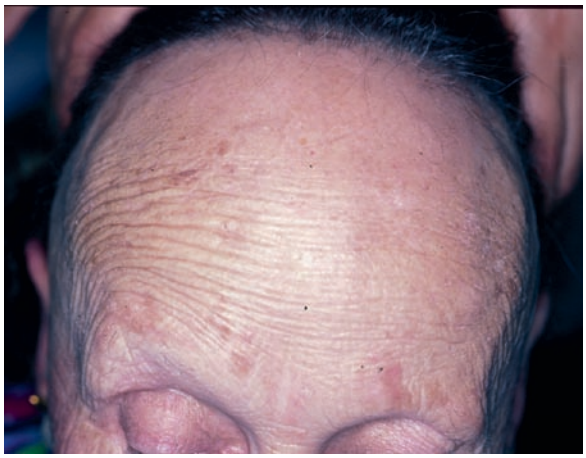

Fig. 4.1 Post-menopausal frontal alopecia with marked recession of hairline, loss of eyebrows and smooth pale skin contrasting with sun-damaged forehead

presenting sign before the recession of the hairline is apparent, but careful examination of the hairline usually reveals perifollicular erythema. Although the main recession occurs over the frontal and temporoparietal areas, the marginal hair loss may rarely extend to the posterior scalp [20]. Uncommonly, the pattern of fibrosing alopecia may produce prominent bitemporal recession with relative preservation of the central frontal hairline (Fig. 4.2). The process is usually asymptomatic, but pruritus may be experienced at the active frontal hairline. FFA has developed uncommonly in premenopausal women [1, 6, 9, 12, 16] and has been reported at a younger age in women who have undergone bilateral oophorectomy [18]. Rare reports of FFA have been documented in men, including loss of eyebrows [21] or complicating androgenetic alopecia [26]. FFA has also been observed in a man who had received hair transplant to the anterior scalp for androgenetic alopecia [11]. Darkening of hair colour adjacent to the band of FFA has been reported in a woman with white hair and PFFA [5].

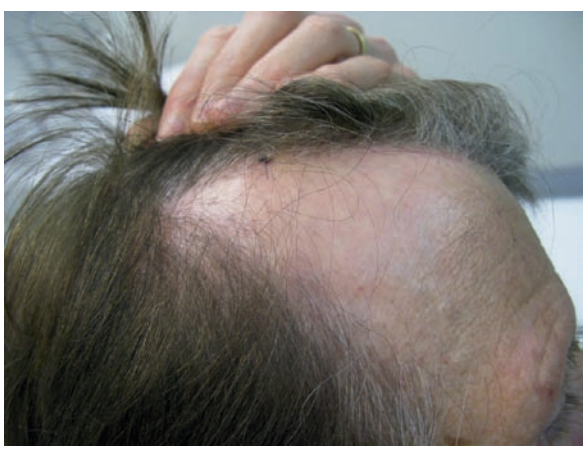

Fig. 4.2 Marked temporal recession with smooth skin due to loss of follicular orifices

FFA usually occurs as an independent entity but can be seen in association with androgenetic alopecia [26] or multifocal lichen planopilaris extending through the scalp [12]. In contrast to lichen planopilaris involving the central scalp, which may have an association with lichen planus at other sites, in up to 50% of cases [14], lichen planus with PFFA has only been reported in occasional patients. Both lichen planus [6] and oral lichen planus [23] have been reported in individual cases and one patient has had lichen sclerosus [7]. PFFA may be associated with asymptomatic hair loss at sites other than the eyebrows including axillae, pubic hair or more generalised hair loss, particularly over the limbs. This pattern of hair loss has been linked with the Piccardi-Lassueur-Graham-Little syndrome [1]. The area of hair loss in such cases has been viewed as being non-scarring and often equated to alopecia areata on clinical grounds and is also a fibrosing hair loss. PFFA usually follows a slowly progressive course but eventually stabilises. However, the severity may be marked with frontal recession up to 8 cm and may prompt the purchase of a wig.

4.3 Histopathological Features

Scalp biopsies from the active edge of hair loss reveal changes that are identical to those seen with lichen planopilaris [2, 12, 15–17, 20, 24]. The main findings are a decreased number of follicles, which are replaced by fibrous tracts, and the remaining follicles have prominent concentric fibrosis around the infundibular, isthmus and canal portions of the hair follicles corresponding to the fully keratinised internal root sheath (Fig. 4.3). These areas are targeted by lymphocytic inflammation that is particularly present outside the ring of concentric fibrosis but are also evident within the follicular sheaths (Fig. 4.4) and at the follicular junction resulting in apoptosis of keratinocytes and eventual destruction of follicular sheaths.

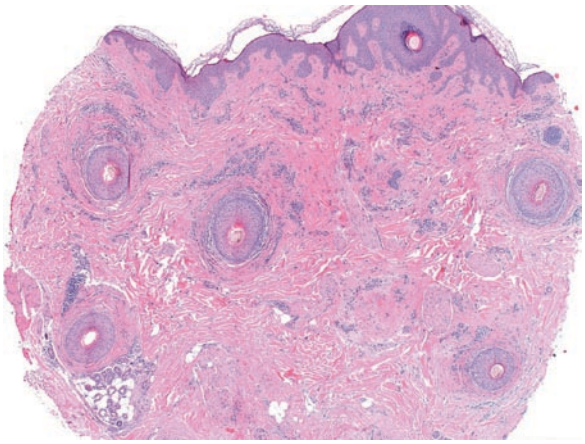

Fig. 4.3 Scalp biopsy from receding hairline demonstrating loss of follicles, perifollicular lymphocytic inflammation and fibrosis (H&E, × 25 magnification)

The interfollicular epidermis in scalp biopsies is usually spared in PFFA in contrast to lichen planopilaris affecting the general skin. One study has found that the lymphocytic reaction targets more small and intermediate-sized follicles relative to the terminal follicles [22], but this is not a universal finding and may be dependent on the time and site of sampling. Early loss of sebaceous glands has been observed in association with lichen planopilaris and PFFA even in adjacent clinically unaffected follicles [15]. Biopsies of established areas of total alopecia reveal loss of all follicles and replacement by fibrous tracts. This represents the end stage of fibrosing alopecia that can be induced by different pathogenic events, particularly lichen planopilaris. Immunophenotyping of the lymphocytes targeting the follicles have revealed a mixture of activated T-cells both of CD4 and CD8 type in ratios similar to that found in lichen planopilaris [10, 12].

The term *fibrosing* alopecia rather than scarring alopecia was initially chosen to describe this entity [10] to reflect the targeted pattern of hair loss seen on biopsy, where follicles are replaced by fine vertical fibrous tracts (immunological epilation) and gross fibrosis in the surrounding dermis is absent. The subtle vertical tracts of fibrosis reflect the clinical presentation of a skin that appears pale and smooth without gross evidence of scarring. Diffuse scaling, erythema and pigmentary changes are absent owing to the sparing of the interfollicular epidermis observed on scalp biopsy and contrasts with the findings seen with lupus erythematosus.

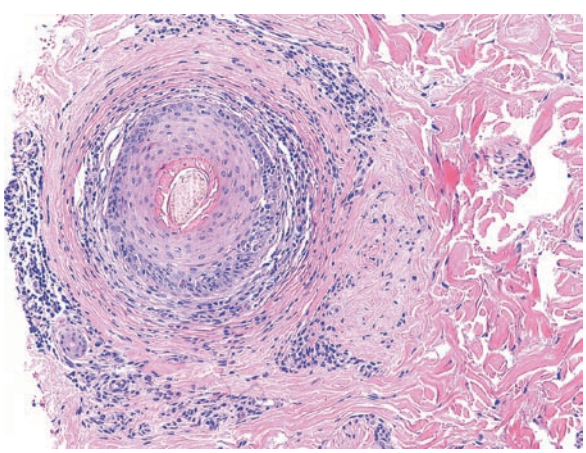

Fig. 4.4 Detail of perifollicular inflammation in a lichenoid pattern and perifollicular fibrosis (H&E, ×100 magnification)

4.4 Laboratory Investigations

Laboratory investigations in PFFA have not revealed any significant abnormalities [2, 12, 16, 22, 24]. Normal investigations have included complete blood count, thyroid function tests and normal sex hormone levels. Antinuclear antibodies have been detected in low titres in some women but antibodies to extractable nuclear antigens or native DNA have not been detected.

4.5 Differential Diagnoses

FFA has distinctive clinical features but the main differential diagnoses may include:

4.5.1 Genetically High Frontal Hairline

Usually, this has an earlier age of onset and is usually familial and does not have a rapid accelerated course. Perifollicular erythema at the hair margin or loss of eyebrows is also not a feature.

4.5.2 Androgenetic Alopecia

The symmetrical pattern and progressive frontal recession are features that are seen with androgenetic

alopecia particularly in men. In the majority of women, the frontal hairline is preserved with female pattern hair loss. In women with late post-menopausal and senescent onset of pattern alopecia, loss of the frontal hairline may also occur [26] but the progressive recession is not associated with total loss of follicular markings and perifollicular erythema at the receding hairline or loss of eyebrows is absent.

4.5.3 Alopecia Areata

Symmetrical frontal hair loss associated with loss of eyebrows may be seen with alopecia areata [13]. In patients with rapid onset of PFFA and loss of eyebrows, this may be misdiagnosed as alopecia areata. Usually, in alopecia areata there is a history of prior episodes with hair regrowth. Perifollicular erythema at the hair margin and loss of the follicular orifices are important clues for PFFA. The ophiasis pattern in alopecia areata has a predilection for the parietal and occipital areas of the scalp in contrast to FFA that is concentrated in the frontotemporal areas.

4.5.4 Lupus Erythematosus

Lupus erythematosus can rarely present as a frontal alopecia. Usually, the areas of hair loss are associated with dyspigmentation, scarring and scaling. Lipoatrophy may also be seen. Lupus erythematosus often has additional cutaneous lesions in sun-exposed sites. Scalp biopsy, immunofluorescence and serological tests for lupus erythematosus may also help to distinguish the two presentations in equivocal cases.

4.5.5 Traction Alopecia

Chronic traction may result in hair loss but usually this is partial and maximal over the temples. The hair loss is gradual and the frontal hairline may be preserved anterior to a band of hair loss due to traction. The majority of women with FFA deny having used hairstyles producing traction and a history of eyebrow plucking is usually not elicited.

4.6 Management

Unfortunately, an effective therapy for FFA has not emerged [12, 16–18, 24]. In the initial rapid hair loss phase, oral corticosteroids may be indicated but most women present with established FFA that slowly progresses. The two major measures of persistent activity are the measurement of the depth of recession and the presence of perifollicular erythema at the receding hair margin. Treatments have included topical or intralesional steroids, topical tacrolimus, topical pimecrolimus or topical minoxidil, oral hydroxychloroquine or oral retinoids, all of which have had no convincing benefit. In one study, eight patients with FFA were treated with finasteride and four showed arrested progression after 12–18 months therapy [22]. The natural course of FFA is usually that of stabilisation, and further evaluation of finasteride is needed as this drug has the potential to modify androgen-sensitive follicular loss that may play an initiating role in post-menopausal women even in the absence of elevated androgens. Finasteride and antiandrogens may be useful particularly in women who have co-existing female pattern hair loss [16].

PFFA has developed in women who are on oestrogen hormone replacement therapy and this does not appear to alter the course of the alopecia [12].

4.7 PFFA and the Ageing Follicles

PFFA remains a puzzling and distinctive presentation of alopecia and has multiple aspects that are difficult to integrate but form clues to its pathogenesis.

4.7.1 Senescent Alopecia and PFFA

As PFFA is seen mainly in elderly women, the relationship to senescent alopecia is relevant to our understanding of PFFA, as the onset may be linked with events that evolve within ageing hair follicles. Senescent alopecia is a universal phenomenon and is associated with a gradual and diffuse loss of follicular density affecting the ageing scalp. In addition to the common diffuse loss of density, senescent alopecia appears to also be expressed as pattern alopecia both in

women [19] and men. The events resulting in permanent senescent hair loss remains unclear but may include progressive miniaturisation and follicular involution and also programmed hair follicle destruction. This programmed loss mediated by lymphocytes targeting effete follicles may be insidious and associated with scattered single follicle loss or may hypothetically be expressed as a sudden and synchronous event as observed in PFFA.

4.7.2 Androgenetic Alopecia and PFFA

Androgenetic alopecia has an early onset and also a late onset form that commences in middle age and progresses as a pattern alopecia that has a distinct distribution in men and women progressing with age. Marked frontal and temple recession is a hallmark of male pattern alopecia, but in women the frontal hairline is preserved. However, in post-menopausal women, frontal and particularly bitemporal recession may develop in the presence of normal levels of androgens [25]. Although PFFA is associated with a striking recession of the frontal scalp, this often extends to the parietal areas that are usually spared in androgenetic alopecia; eyebrow loss is also a distinctive feature. These features indicate that even if the initial event of post-menopausal frontal recession is responsible for triggering the lymphocytic follicular destruction, the subsequent course results in a pattern of hair loss, which is clearly not confined to the areas seen usually with androgenetic alopecia.

4.7.3 Fibrosing Alopecia and Pattern Hair Loss

The prototype of fibrosing alopecia is lichen planopilaris that represents a lymphocyte-mediated targeted destruction of follicles. The lymphocytes target the permanent infundibular and isthmus portions of the follicles as well as the fully keratinised canal portion that include the stem cell rich areas. Scalp biopsies from PFFA have regularly shown a pathology, which is essentially indistinguishable from lichen planopilaris.

The development of diffuse fibrosing alopecia confined to the areas of pattern hair loss have been recently described in both men and women [19, 26]. Careful examination of the balding scalp in these individuals shows perifollicular erythema and progressive permanent loss of follicles with loss of follicular openings. Scalp biopsies revealed identical features to those observed with lichen planopilaris and in PFFA. A multifocal form of hair loss without perifollicular erythema but with small pale areas of focal atrichia has also been observed in women and termed cicatricial pattern hair loss [19].

4.7.4 PFFA and Pattern Hair Loss

PFFA has developed in the setting of pattern alopecia, but in some women, the fibrosing alopecia may be confined to the marginal scalp line though the co-existing pattern alopecia over the central scalp shows no evidence of diffuse or focal fibrosing alopecia [12, 24]. Furthermore, in rare cases, women may develop multifocal hair loss over the central scalp typical of classical lichen planopilaris and also the more diffuse and symmetrical marginal hair loss of PFFA [12].

One of the intriguing features of FFA is the extreme rarity of this presentation in men as an initial presentation of pattern alopecia when compared with elderly women. The key to this may be the difference in pattern alopecia in men and women with regard to the preservation of the frontal hairline. The rarity of FFA in men may be due to the fact that frontal and temporal recession occurs regularly with male pattern hair loss at an earlier age that may not be associated with the risk of stimulating an autoimmune reaction to involuting hair follicles. The frontal hairline appears to be protected in women but can undergo recession after the menopause [25] and with senescence. This particular form of late pattern recession in post-menopausal women may play a role in initiating an autoimmune response in the setting of a sudden change in follicular receptors that are influenced by androgens at a time of senescence and lead to a breakdown of immune protection of the follicles. The role of hormones, particularly estrogens, remains uncertain as HRT does not appear to influence the onset of PFFA, and hormone studies so far have not been helpful in addressing this

issue. The changes, however, may be programmed in the frontal follicles. There are, however, aspects that do not tie in well with this hypothesis, as PFFA often strikes suddenly in women who have no evidence of preceding pattern hair loss. In addition, the loss of eyebrows and the often prominent recession of the parietal hairline are features that are not part of female or male pattern alopecia.

4.8 Conclusions

PFFA is an alopecia that is dominated by lymphocyte-mediated targeted follicular destruction and currently is best classified as a subset lichen planopilaris. The basis for the varied presentations of lichen planopilaris is still a matter for investigation. Further work is required to determine why the striking patterned hair loss in PFFA is seen mainly in elderly women. It remains to be seen whether events linked with the menopause, senescence or changes in the immune status of follicles in the frontal hairline trigger this autoimmune reaction. This synchronous follicular destruction by lymphocytes may induce an expanded hair loss that has its own distinctive pattern not seen with androgenetic hair loss and recognised as PFFA.

Take Home Messages

- PFFA is an increasingly recognised form of permanent pattern recession due to lymphocyte-mediated follicular destruction.
- Although this alopecia is also seen occasionally in premenopausal women and rarely in men, there is a distinct predilection for postmenopausal elderly women.
- Currently, the pathogenesis of this alopecia remains unknown but appears to be triggered by events at the frontal hairline in women that may be linked with menopause and senescence and loss of follicular immune privilege that triggers an autoimmune targeted destruction of follicles in a patterned distribution.

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Female Pattern Hair Loss

5

M. Javed Mohungoo and Andrew G. Messenger

Core Messages

- Female pattern hair loss typically presents with a history of gradual thinning of scalp hair and widening of the central parting.
- The prevalence and severity increase with age.
- Hair loss is due to a gradual decline in the production of hair by scalp hair follicles culminating in follicular miniaturization.
- Androgens are an important factor in driving follicular miniaturization but it is likely that other nonandrogenic mechanisms are also involved.
- Medical treatment can help to prevent progression of hair loss and may promote modest regrowth of hair but is probably unable to reverse follicular miniaturization.

5.1 Introduction

Female pattern hair loss (FPHL) is a common condition in women characterized by a progressive decline in scalp hair density. It is also known as female androgenetic alopecia, a term first used in 1960 by Orentreich [26], suggesting that it is the female equivalent of male androgenetic alopecia. Male androgenetic alopecia is a genetically determined androgen-dependent trait [10]. The role of androgens in female hair loss is less clearcut than in male balding; hence, the use of the less committal term "female pattern hair loss" has gained currency and is used in this review. The prevalence and severity of both FPHL and male androgenetic alopecia increase with advancing age, suggesting that their etiology also includes age-related factors (Fig. 5.1).

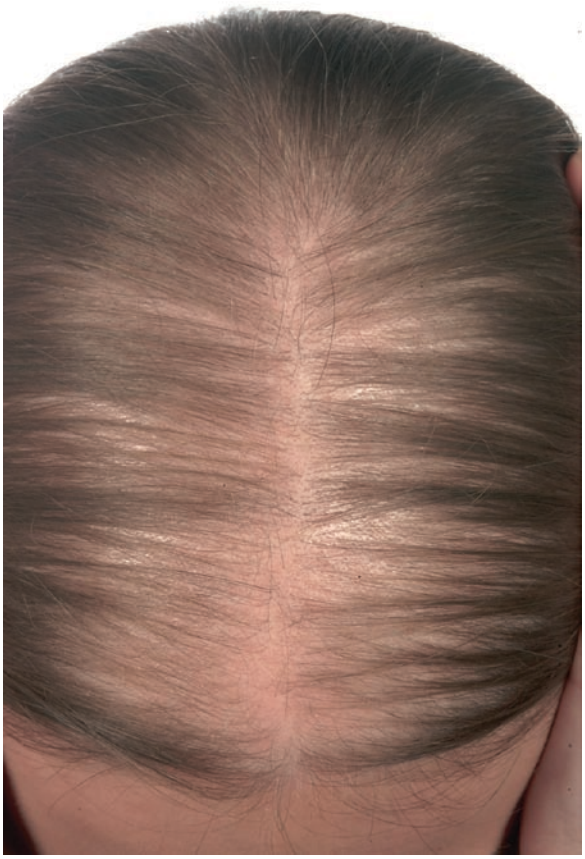

Fig. 5.1 Female pattern hair loss (FPHL)

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5.2 Clinical Features

In a short but influential paper in 1977, Ludwig described the distinctive features of FPHL and classified it into three grades of severity, now known as Ludwig I, II, and III [20]. This classification is still largely in use to this date.

Most women with FPHL present with a history of gradual thinning of scalp hair over a period of several years [20]. There is sometimes a history of excessive hair shedding, which can predate a clinically obvious reduction in hair density. Examination of the scalp shows a diffuse reduction in hair density with widening of the central parting. Hair loss is usually most pronounced in the mid and frontal regions of the scalp, but can range from involvement of a small area of the frontal scalp to affecting the entire scalp including parietal and occipital regions. However, the frontal hairline is typically retained even though some women develop a minor degree of postpubertal recession at the temples, with or without diffuse hair loss. The onset can be at any age starting from puberty.

5.3 Prevalence

The population frequency and severity of FPHL increase with age. Two studies in Caucasian women in the United Kingdom and United States reported prevalence rates of 3–6% in women aged under 30, increasing to 29–42% in women aged 70 and over [2, 23]. The frequency is lower in oriental women [28] (Fig. 5.2).

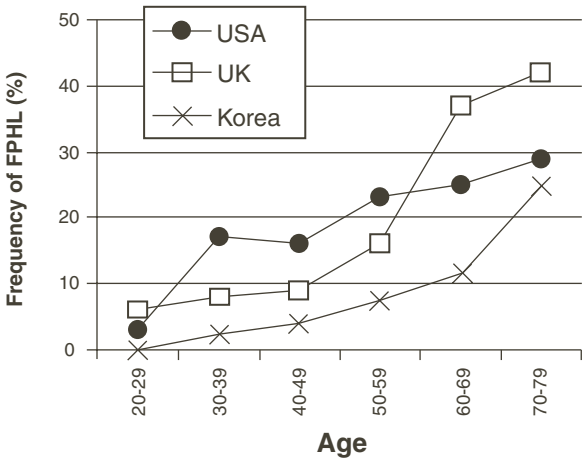

Fig. 5.2 Age-related prevalence of FPHL in the USA, UK, and Korea

5.4 Pathology

Pattern hair loss is due to a decline in the production of hair by hair follicles. The duration of anagen becomes shorter and that of telogen lengthens. The time the telogen club hair is retained within the follicle probably remains the same but reentry into anagen is delayed. Follicles then undergo a regressive process – miniaturization – which may eventually lead to their deletion. The histological hallmark of pattern hair loss in both sexes is an increased proportion of small "vellus-like" follicles. This is manifested as an increase in the absolute number of vellus follicles and a decline in the ratio of terminal:vellus follicles. In normal scalp, the terminal:vellus ratio is typically in excess of 8:1, whereas in established pattern hair loss, it is no more than 3:1 [42]. In early pattern hair loss, the total number of follicles is within normal limits, but as hair loss advances, this falls [21]. There is also an increase in the proportion of follicles in telogen due to the shortening of the anagen phase of the hair cycle and a lengthening of telogen. A mild-to-moderate chronic inflammatory infiltrate around follicular infundibula is a common finding, although this may also occur in normal scalp. Perifollicular fibrosis may be seen in the later stages of pattern hair loss [16, 41].

Follicular miniaturization is conventionally regarded as a gradual process, occurring over the course of several hair cycles. This has yet to be demonstrated convincingly in longitudinal studies and there is some evidence that miniaturization occurs rapidly, possibly within the space of a single hair cycle [2].

5.5 Etiology

5.5.1 Age

Age is clearly an important factor in pattern hair loss but whether the follicular regression seen in pattern hair loss involves the molecular and cellular events linked to tissue aging is not yet known.

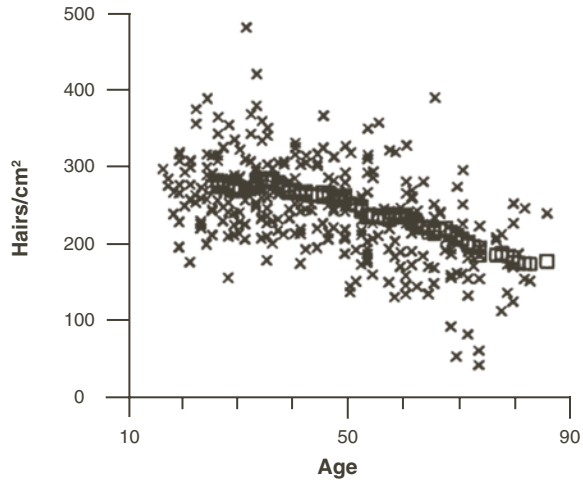

Fig. 5.3 The distribution of hair density by age in an unselected sample of 377 women

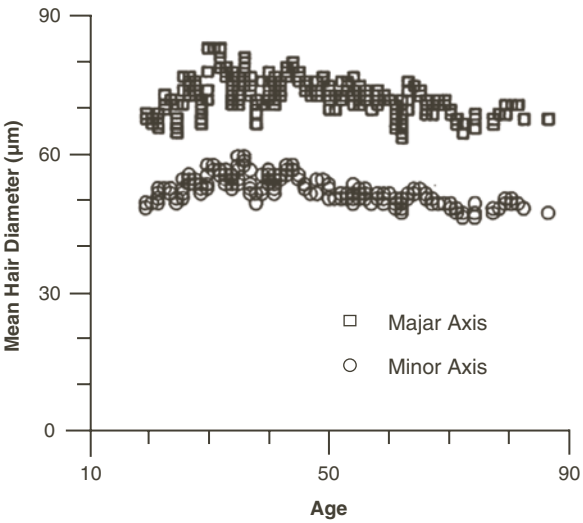

Fig. 5.4 The distribution of hair diameter with age

Cross-sectional data show that from around the age of 30 hair density in the female population declines gradually with advancing years (Fig. 5.3). Mean hair diameter also falls over a similar age range [2] (Fig. 5.4). These data show no evidence that the decline in hair density and hair diameter accelerates following the menopause. Longitudinal data derived from a group of men studied over a period of several years showed that the duration of anagen declines with increasing age [6]. The duration of telogen up to the release of the club hair (exogen) remained constant over the course of the study but the latent period, also known as kenogen, the time from telogen release until onset of the next anagen, becomes longer [9]. These changes were more pronounced in men with androgenetic alopecia. Similar studies have not been done in women, although it is reasonable to assume that the same events occur and there is some evidence that FPHL is associated with the prolongation of kenogen [32]. We would therefore expect that increasing age is associated with a decline in the final length of an individual hair shaft owing to the shortening of anagen and an increasing delay in its replacement following shedding. The latter would result in an "empty" follicle and contribute to the fall in hair density. These changes appear to be exaggerated in pattern hair loss raising the question as to whether pattern hair loss involves a premature expression of normal age-related mechanisms.

The additional feature in pattern hair loss is follicular miniaturization [21], but it is possible that this merely represents the end point of a regressive process that occurs in most of us as we age, but which occurs more rapidly in those with more overt hair loss. As in other physiological traits, the boundary between normality and "abnormality" is blurred, suggesting that pattern hair loss is a continuum from nonhair loss rather than a separate defined entity. This is especially true of FPHL, in which minor degrees of hair loss can be difficult if not impossible to detect at the clinical level. In men, the idea that hair thinning – "senile alopecia" –may occur as an age-related process independent of androgens is well established, although the evidence is limited [22]. The term is applied to the more diffuse global reduction in scalp hair density sometimes seen in elderly men. Hair thinning in women with FPHL often extends to regions not typical of androgenetic alopecia, such as the parietal and occipital scalp, suggesting that it is more akin to male senile alopecia than to male androgenetic alopecia. Thus, androgen-independent aging mechanisms may play a more important role in FPHL than in men.

5.5.2 Androgens

The role of androgens in male pattern hair loss is incontrovertible. Scalp hair loss is also undoubtedly a feature of hyperandrogenism in women. Indeed, case reports of hair loss in women with virilizing tumors [1, 17]

predated Hamilton's seminal observations in men [10]. Several studies have reported that women with hair loss are more likely to have elevated androgen levels or show an increased frequency of other features of androgen excess than women without hair loss. Futterweit and colleagues studied 109 women with hair loss and reported that 38.5% showed clinical or biochemical evidence of androgen excess [8]. In a series of 187 women with hair loss, Vexiau et al reported abnormal hormonal profiles, mostly of minor degree, in 67% of women with hair loss alone and in 84% of women who were also hirsute [40]. In a series of 89 women presenting to a trichology clinic with hair loss, 67% showed ultrasound evidence of polycystic ovaries when compared with 27% in a control group of 73 women, and 21% were significantly hirsute when compared with 4% of controls [5]. However, other investigators have failed to find evidence of raised androgen levels in women with FPHL [34] and in all studies there is a variable proportion of women with hair loss who do not show clinical or biochemical signs of androgen excess.

5.5.3 Genetics

It has long been known that male balding runs in families. Less is known of the inheritance of FPHL but published genetic models have generally assumed that male balding and FPHL are the same entity. Osborn proposed that balding in men and in women is due to a single gene with two alleles, *B* (balding) and *b* (nonbalding) [27]. She suggested that balding occurs in homozygous (*BB*) and heterozygous (*Bb*) men but only in homozygous (*BB*) women. However, in a critique of the published data, Kuster and Happle argued that the predisposition to balding is a polygenic trait, in which clinical expression represents a threshold effect [18]. They suggested that the threshold is higher in women due to their lower androgen levels, and women therefore need a stronger genetic component than men for hair loss to occur. In the only published study of family histories in balding women other than that of Osborn, Smith and Wells found that first-degree male relatives showed an increased frequency of balding when compared with the male relatives of nonbalding women [38]. Little is known of the genes responsible for balding. A recent twin study provides strong evidence that the predisposition to balding in men is genetically determined. Three studies have implicated variant regions in the androgen receptor (AR) gene at Xq11–q12 in predisposing to [7, 12] or protecting against male balding [11], and a recent study from Germany identified a susceptibility locus at chromosome 20p11 [13]. Similar studies have yet to be performed in FPHL.

5.6 Management

Although it may be considered as a physiological trait, FPHL is undoubtedly a distressing process for women in a society, which regards this as being abnormal. Consequently, the adverse effect on the quality of life tends to be more severe in women than in men [27].

5.6.1 Diagnosis

The diagnosis of FPHL is usually straightforward. It typically presents with a diffuse reduction in hair density of gradual onset, which is most pronounced over the frontal and mid-portions of the scalp although thinning in the parietal regions is frequently seen and, in contrast to male balding, loss of hair density in the occipital scalp is not unusual. In some women, frontal hair loss is predominant giving rise to the so-called "Christmas tree" pattern [25]. Most retain the frontal hairline, although thinning in the fronto-temporal regions is common.

Rapidly progressing hair loss with a strongly positive "pull test" should raise the possibility of diffuse alopecia areata. Other causes of diffuse hair loss include systemic lupus erythematosus and thyroid disease. The most difficult differential diagnosis is from chronic telogen effluvium [42]. Here, the presentation is with persistent excessive hair shedding and loss of hair volume but with retention of hair density. Most patients are middle-aged women. In some chronic telogen, effluvium is probably the result of age-related changes in hair cycling combined with a high hair density, long hair, and a personality that places a high value on appearance. However, biopsy studies have shown that around 60% of women presenting with chronic telogen effluvium show evidence of follicular miniaturization, indicating they are in the early stages of FPHL [36]. In a further subset, increased hair shedding may be due to diffuse alopecia areata. The idea

that iron deficiency in the absence of anemia is a cause of excessive hair shedding is widely held but, as yet, unsupported by conclusive evidence.

Importantly, FPHL can be a feature of hyperandrogenism and signs such as hirsutism, resistant acne, irregular menses, infertility, and/or galactorrhoea should prompt further investigations. Although hair loss in hyperandrogenism is conventionally associated with a more "male" distribution, it is the authors' experience that the pattern of hair loss is not a reliable guide and typical FPHL does not rule out significant androgen excess. Polycystic ovary syndrome is by far the most common cause of androgen excess, but it is important not to miss an androgen secreting tumor. Measurement of serum testosterone is the single most useful investigation – levels above 5 nmol/L require further investigation and referral to an endocrinologist [23].

5.6.2 Investigation

Most women presenting with typical FPHL do not need to be investigated. It seems reasonable, however, to check the full blood count, thyroid stimulating hormone level, and, in cases where androgen excess is suspected, serum testosterone. A scalp biopsy is seldom required but should be considered in cases where there is diagnostic doubt, for example, in cases where other causes of diffuse hair loss cannot be excluded on clinical grounds. Should a biopsy be taken, transverse sectioning is essential to diagnose FPHL histologically, as well as a histopathologist experienced in the interpretation of transverse sections. We take two 4 mm punch biopsies, usually from mid-frontal scalp lateral to the mid-line. One biopsy is sectioned transversely, the other vertically.

5.6.3 Treatment

Counseling of patients with FPHL should include an explanation of the nature of the condition and its natural history. For those interested in preventing further progression or improving their hair status, the treatment options, together with a realistic explanation of what can be achieved by treatment, will also need to be discussed. Some women are content to be reassured that their hair loss is not a manifestation of a serious disease and that it is very unlikely that they will go bald. For those who are keen to be treated, there are two medical options - minoxidil lotion and antiandrogens. In both cases, it should be stressed that treatment will, at best, produce only a modest increase in hair density and that it is not possible to fully reverse hair loss. Furthermore, in those who respond, treatment has to be continued to maintain the response. As in men, surgery is the only method capable of restoring the appearance in the presence of severe hair loss.

5.6.3.1 Minoxidil

Minoxidil lotion 2% is licensed for the treatment of female androgenetic alopecia in most countries. The 5% formulation is not currently licensed for use in women. Clinical trials in the early 1990s using hair counts as a primary endpoint reported a mean increase in hair growth of 15–33% in the minoxidil-treated groups when compared with 9–14% in the vehicle control groups [15, 24, 43]. One small study using hair weight as the endpoint found an increase of 42.5% in hair weights in the minoxidil group when compared with 1.9% in the controls [30]. In the investigator and subject assessments, minoxidil was superior to the vehicle but about 40% of subjects appeared not to respond to minoxidil [15, 43]. A more recent trial comparing 5 and 2% minoxidil lotion found increases of 18 and 14%, respectively, in mean nonvellus hair counts after 48 weeks treatment, when compared with a 7% increase in the placebo group [19]. As in men, the increase in hair counts following treatment with minoxidil lotion is noticeable within 8 weeks and has peaked after 16 weeks, suggesting that minoxidil acts primarily on the hair cycle.

Minoxidil is a safe treatment. Some patients complain that it leaves unsightly deposits on the hair. Occasionally, it causes scalp irritation, which may be severe enough to cause a temporary increase in hair shedding, and patients should be warned about this. Irritant reactions are more common with higher concentrations of minoxidil. Hypertrichosis on the face and on more remote sites has been reported, particularly when higher concentrations of minoxidil are used [29]. This resolves if treatment is stopped.

In the authors' experience, minoxidil lotion is more reliably effective and better tolerated than anti-androgen treatment. Nevertheless, the gains are modest and it is

helpful to have an objective measure, such as serial standardized clinical photographs, to convince the patient (and the physician) of the response. Once a full response has been obtained (e.g., after 6–12 months), some patients are able to reduce the frequency of minoxidil application, as low as once or twice a week, without an obvious loss of hair density.

5.6.3.2 Antiandrogens

The antiandrogens cyproterone acetate, spironolactone, and flutamide have been used to treat female androgenetic alopecia, as has the 5α-reductase inhibitor finasteride, although none is licensed for this purpose and there is little clinical trial evidence of efficacy for any of them.

Cyproterone acetate acts by blocking ARs. It also has progestational activity and suppresses the production of gonadotrophins. It is not available in the USA but is widely used in Europe, usually in a cyclical regimen in combination with the oral contraceptive Dianette™. In a randomized controlled trial in 66 women with female androgenetic alopecia, cyproterone acetate 52 mg daily plus a combined oral contraceptive was compared with minoxidil lotion 2% [40]. After 12 months treatment, nonvellus hair density increased significantly in the minoxidil-treated group but fell in the cyproterone acetate group. However, subgroup analysis showed a small improvement in hair density in women with menstrual irregularities receiving cyproterone actetate. This study suggests that antiandrogens may be beneficial in women with evidence of androgen excess but not in those without – a conclusion in keeping with the personal experience of the authors.

Spironolactone is a competitive inhibitor of aldosterone receptors. It also blocks ARs and increases metabolic clearance of testosterone. It has been widely used to treat hirsutism. There are no controlled trials of its use in female androgenetic alopecia. Rushton and colleagues reported that women treated for 12 months with spironolactone showed less hair loss than an untreated group [33]. In an open uncontrolled case series of 80 women treated for 1 year with spironolactone (200 mg daily), or cyproterone acetate, 35 (44%) showed improvement in hair growth as assessed by standardized photography [37].

Flutamide is a pure AR blocker. A randomized trial from Italy compared flutamide 250 mg daily with cyproterone acetate and finasteride in the treatment of 48 hyperandrogenic women with androgenetic alopecia. Those treated with flutamide showed a modest improvement in hair growth, whereas those treated with cyproterone acetate or finasteride did not [3]. The study appears not to have been blinded and the method of assessment, using the Ludwig grading system, was relatively crude.

In a large randomized controlled trial in postmenopausal women with androgenetic alopecia, finasteride 1 mg daily proved ineffective in preventing hair loss [31]. However, improvement has been reported in a small case series of hyperandrogenic women [35] and in a larger series of 37 premenopausal women treated for 1 year with finasteride 2.5 mg daily [14]. In the latter study, 62% showed some improvement as assessed by global photography.

Antiandrogen treatment is not without problems. As with minoxidil, treatment has to be continued to maintain a response and women taking antiandrogens should not become pregnant because of the risks of feminizing a male fetus. Dose-related side-effects of cyproterone acetate, including weight gain, fatigue, loss of libido, mastodynia, nausea, headaches, and depression, are common. There is a significant risk of hepatotoxicity with flutamide and cyproterone acetate is also potentially hepatotoxic in high doses. Spironolactone may cause breast soreness and menstrual irregularities but is probably the safest option and is the personal preference of the authors. Finasteride is well tolerated and is worth considering in postmenopausal and infertile women.

5.6.3.3 Surgery

Hair transplantation is less widely used than in men but can give good results in selected cases and is the only treatment that can produce a substantial improvement in those with advanced hair loss [39]. It is most appropriate in women with pronounced hair loss of limited extent who retain good hair density in the donor site. Those with a mild degree of hair loss are less suitable as are those with the involvement of the occipital region.

5.6.3.4 Cosmesis

A variety of cosmetic measures can help to improve appearance in FPHL. At the simplest level, good hair

care and a skilled hairdresser can help to minimize the deficit in hair density. Some women are helped by topical applications that coat hair shafts to increase the impression of hair volume. For advanced hair loss prosthetics, such as wigs, extensions or weaves may be needed. Very realistic results can be achieved by skilled practitioners, although cost is often a limiting factor for the patient.

5.7 Summary

FPHL may be considered as a normal biological process. Nevertheless, it can be the source of great anxiety and psychological upset. The origin of the adverse effects on the psyche are undoubtedly complex but include the widely held view that it is abnormal for women to lose their hair, the perception that scalp hair loss is a "masculine" trait and, despite the fact that FPHL can start in early teens, that it is a manifestation of aging. Although some of these ideas are mistaken, age is clearly an important factor. Follicular miniaturization may be a final common pathway of follicular regression, the end result of a multifactorial process. We do not yet know whether aging mechanisms form part of this process but in view of the contribution of age to the prevalence and severity of FPHL it seems likely to be the case.

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Hair Growth Parameters in Pre- and Postmenopausal Women

6

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Core Messages

› Women who undergo menopause have a cessation of ovarian estrogen production. This dramatic hormonal alteration is known to have significant effects on the skin and cutaneous appendages. As our understanding of the molecular and hormonal controls on the folliculo-sebaceous unit has grown, there has been renewed interest in the role of estrogens in modulating hair growth. Specifically, the relatively recent discovery of estrogen receptor beta has broadened and redefined prior concepts of estrogen activity and signaling. In a cohort of pre- and postmenopausal women without alopecia, a modified phototrichogram was used to measure hair density, growth rate, and percentage anagen. Optical fiber diameter analysis (OFDA) was used to determine hair diameters. Our aim was to determine whether there are any changes in hair characteristics and hair growth parameters that correlate with menopausal status. Postmenopausal women had significant changes, mainly in the frontal scalp as compared to premenopausal women. These changes included lower frontal scalp percent anagen hairs, growth rates, and hair diameters. Further study of hair changes in response to menopause provides an important opportunity for identification of treatments, targets, and strategies that may significantly benefit women.

6.1 Introduction

A variety of techniques are available for assessing hair characteristics. These measurements include hair density, growth rate, anagen/telogen ratios, diameter, weight, tensile strength, and curvature, among others [1 2, 32]. Development, validation, and refinement of the tools to measure these variables have occurred in large part due to an interest in scientifically assessing the efficacy of hair growth modulators [24]. In this chapter we discuss the use of novel techniques to measure hair characteristics of pre- and postmenopausal women without alopecia. The goal of these investigations was to determine whether there are changes in hair characteristics and hair growth parameters that correlate with menopausal status.

6.1.1 Menopause

Menopause is either defined as the permanent cessation of menses or the lack of menses for 12 consecutive months [29]. The mean age women undergo menopause is 51, thus women spend about one-third of their life in the postmenopausal period [29]. One of the major

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changes during menopause is the near cessation of ovarian estrogen production. The major source of estrogen after menopause is from conversion of adrenal androgen to estrogen by the enzyme aromatase in the peripheral tissues. While it has been recognized that estrogen is an important modulator of hair growth, the details of the molecular regulatory pathways have not been well-characterized. In contrast, the role of androgens on hair growth has dominated the field of hair biology. As our understanding of the molecular and hormonal controls on the folliculo-sebaceous unit has expanded, there has been renewed interest in the role of estrogens as well as androgens in modulating hair growth.

6.1.2 Estrogens and the Hair Follicle

Estrogen is synthesized in the ovary as well as in a number of peripheral tissues and acts via estrogen receptors which belong to a superfamily of nuclear receptors. There are two estrogen receptors, alpha (ER alpha) and beta (ER beta). The relatively recent discovery of ER beta has broadened the range of potential estrogenic target tissues and has also redefined prior concepts of estrogen activity and signaling. In the human hair follicle, immunohistochemical studies have shown ER beta to be the predominant receptor [36, 37]. Similar to other estrogenic target tissues, the biologic activity of estrogen in the hair follicle likely depends on a complex interplay of signals that may differ depending on the relative distribution and location of the two ERs, as well as the activity of the peripheral converting enzyme, aromatase [7 21, 22]. Several studies have demonstrated the influence of estrogen on the murine and other mammalian hair cycle; however, it is clear that the distribution, expression, and biologic activity of estrogen receptors in murine models may be quite different than in humans [5, 16, 19–21, 31, 33]. Indeed, in a recent study, a topical antiestrogen that caused hair growth in a murine model was ineffective in humans [9]. In vitro studies have shown that organ culture of human scalp hair follicles exposed to estradiol results in decreased growth whereas cells of the dermal papilla responded with proliferation [7, 12]. Estradiol has also been noted to induce aromatase activity in human scalp follicles, one possible mechanism by which it may exert biologic activity [10]. Since hair growth is influenced by numerous hormones, growth factors, transcription factors, and cytokines, many of which are known to be modulated by estrogens, it is plausible that an intricate orchestration of these pathways occurs in response to estrogen. Further clarification and study of estrogen effects in different tissues, species, and sexes is ongoing. Since a detailed discussion of the molecular pathways of estrogens in the hair follicle is beyond the scope of this chapter, the reader is referred to a number of excellent reviews of this topic [6, 22, 35].

6.1.3 Clinical Observations

Various clinical observations have supported the notion that estrogen is an important regulator of hair follicle growth and cycling. The use of topical estrogens to treat androgenetic alopecia and telogen effluvium has been proposed based on small clinical studies and is commercially available in Europe, but their efficacy remains controversial [10, 26] The use of oral contraceptives, their cessation, or hormone replacement therapy, have all been reported to cause a temporary hair shedding or telogen effluvium [2]. Although the progesterone component of oral contraceptives has been thought to be the culprit in such an effluvium, the estrogen content may also have an effect. Women with breast cancer who take medications aimed at decreasing the effect of estrogens (selective estrogen receptor modulators), or medications that block the peripheral production of estrogen (aromatase inhibitors) commonly report hair loss. In pregnant women, there is an increase in hair growth rate, diameter, and the ratio of anagen/telogen hairs [15, 17 18]. Although higher estrogen levels likely play a role in this alteration of hair biology, it is difficult to attribute the phenomenon to estrogen alone because of the complex hormonal changes seen in pregnancy including increases in progesterone and prolactin. Chronic telogen effluvium is another condition that results in diffuse scalp hair loss and is typically seen in women in their fourth to sixth decades of life. It typically presents with an abrupt hair shedding that can have a fluctuating course lasting at least 6 months and may continue for 6–7 years. Although chronic telogen effluvium is usually self-limiting, the volume of hair typically does not return to premorbid volume. It has been proposed, but not confirmed, that decreasing estrogen levels may contribute to this clinical phenomenon. The inflammatory hair disorder frontal fibrosing alopecia (FFA) was

reported to occur in a higher rate in postmenopausal women. However, hormone replacement therapy did not appear to alter the course of disease. After the initial description, FFA has also been reported to occur in premenopausal women and men; therefore, the role of menopause and specifically estrogen may not be as significant in FFA as originally thought.

It has been suggested that the various clinical patterns of androgenetic alopecia in men and women may reflect quantitative differences in levels of androgen receptor and steroid-converting enzymes in specific scalp regions at different ages [30]. However, in recognition that there may be other nonandrogen causes of hair thinning in women that have no counterpart in men, the term female-pattern hair loss (FPHL) has been coined to encompass the clinical phenotype of hair loss in the central scalp region that may occur: (1) in genetically predisposed women due to androgens; (2) nonhereditary androgen excess; (3) hormonal changes due to menopause [23, 25]. Although the etiology of FPHL has not been fully elucidated, understanding the many hormonal factors that are changing in concert during middle age and menopause may be helpful in this group. A study supporting the notion that nonandrogen pathways may be involved in FPHL showed that sebum secretion, a marker of end organ androgen response, was not elevated [4]. The report of a woman with hypopituitarism and hair thinning in the absence of detectable androgen levels also suggests that hair thinning is not exclusively androgen-dependent [27].

Further complicating our understanding of the possible role of menopause on hair characteristics is whether age-related changes may coexist, overlap, or supercede hormonal changes. Various reports have suggested that decreased hair density and diameter occurs with advancing age in both sexes [3, 8, 11, 13, 34].

In summary, there is convincing evidence from clinical observations that menopause and specifically estrogens have an effect on hair biology. However, there is currently inadequate information to attribute specific hair changes to hormonal alterations seen in menopause.

6.1.4 Measurements of Hair Growth Parameters

One of the major advances in the techniques for measuring hair growth parameters has been the use of the phototrichogram. This technique involves photography of a target scalp area in which the hair is clipped to approximately 1 mm in length. A repeat photograph of the target area is taken 1–3 days later. Of the clipped hairs, only those in anagen will grow during this time. With this method, data can be obtained on follicular density and hair diameter, as well as the percentage of hairs in anagen and the anagen/telogen ratio. Further enhancement of the phototrichogram technique has included the use of digital luminescence microscopy which flattens the hair through direct contact with the scalp, thus allowing for hair length measurement and determination of growth rate. In addition, the use of a contrast-enhanced phototrichogram has enabled improved detection of finer caliber hairs and this noninvasive technique has been shown to be as reliable as scalp biopsies. The measurements on the phototrichogram may be done manually, by computer, or by a combination of the two (computer-assisted), each of which has its benefits and drawbacks. As hair follicles are not evenly distributed on the scalp, repetitive capture of the same set of hairs is crucial over time. To accomplish this, some sort of mark must be placed on the target area for repositioning. For single event experiments, such as was done in the studies mentioned here, a temporary dot made with a permanent ink or Henna pen may be used, but when hair characteristics are being measured over time, a tattoo is highly recommended. In order to facilitate adequate repositioning to measure the subtle changes present in menopause, we developed a new "real time" image capture method.

The technique that we used to measure the hair characteristics of the pre- and postmenopausal women described in this chapter is a modified phototrichogram that employs a process termed "blending" to produce a montage photograph of the baseline and follow-up visits. As previously reported, a Hi-Scope fiber-optic remote head microscope probe was used with a ×25 magnification lens to digitally capture and store scalp target area photos. At the follow-up visit the fiber-optic probe was repositioned at the target area to achieve nearly precise alignment or overlap with the baseline image so that the characteristics of each follicular unit could be identified (Fig. 6.1). Manually performed hair identification and computer-assisted hair measurements were performed, yielding number density, growth rate, and anagen status.

In addition to the modified phototrichogram analysis, hair diameter measurements were made on our

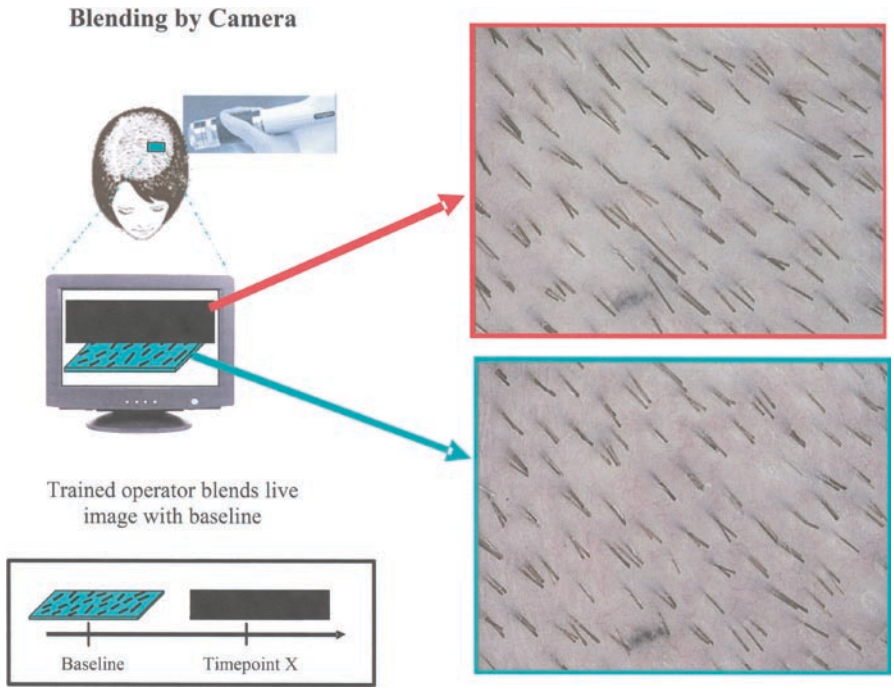

Fig. 6.1 Blending by camera manipulation

initial cohort of pre- and postmenopausal women (initial study) as well as a second, larger, cohort of women (extended study). Although hair diameter can be measured on magnified images of hairs captured on phototrichograms, we utilized a new technique adapted from the wool industry. Optical fiber diameter analysis (OFDA) is a highly sensitive and reliable technique for measuring hair diameters that was initially optimized and validated in the wool industry and has now been adapted for research purposes in human hair. The OFDA instrument analyzes electronic images of horizontally cut and magnified hairs which are distributed over a glass slide. Software analysis of these images derives large volume, rapid, and highly accurate measurement of diameter of various longitudinal hair sections [14, 28]. In addition to the significant speed and ease of OFDA measurements, another advantage of this technique is generation of data on the distribution of hairs as a function of diameter (Fig. 6.2).

Briefly, the OFDA method makes hundreds or thousands of measures of individual hair "snippets" by image analysis of fragments spread on a glass slide. A sample of hair is taken as close as possible to the scalp and the proximal 2 mm is snipped off in a special cutting device. This allows measurement of the hair diameter from all of the individual hairs in the sample – enabling analysis of the diversity of the populations of hairs. Most standard techniques (Diastron, embedding for cross-sectional area, or weight/length with density) for hair diameter measurement are too labor-intensive to analyze more than a few hairs from any individual sample. Using OFDA, we have found that human hair contains many "modes" or different populations of hairs in a single individual. The OFDA measurement enables looking at the overall diameter of the population in a single sample, comparing the number of modes, the proportion of each mode, and also the indi-

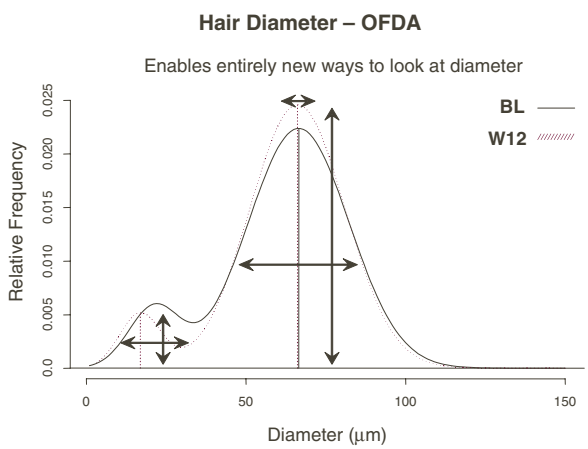

Fig. 6.2 OFDA distributions of hair diameter

vidual diameters and variability of each mode. An example of the distributions is shown in Fig. 6.2.

In development of OFDA for human hair measurement, we compared OFDA measures to a set of other analyses from the identical hair bundles. These measures included diastron long and short axis and calculated cross-sectional area, average diameter calculated by weight of specific numbers of hairs of specific lengths, and embedded cross-sectional area. The specific results of this analysis are reported elsewhere, but overall, the OFDA mean hair diameters were shown to correlate with mean long axis of the hair and capture a significant hair diameter distribution from 5–25 μm. Examples of the distribution on single subjects and the diversity of hair diameters are demonstrated in Fig. 6.3. The extremely high diameter diversity revealed by OFDA shows how difficult it can be to understand subtle changes in hair diameter due to aging, hormonal status, or pharmacologic intervention.

6.2 Subjects and Methods

Women ages 18 or older without any significant hair loss or history of hair or scalp disorder were eligible. All women filled out a brief questionnaire regarding their menopausal status as well as use of any medications that might alter hair growth. Women with gray hair had hair dye applied to the target area prior to photographs. Hi-scope phototrichograms with blended

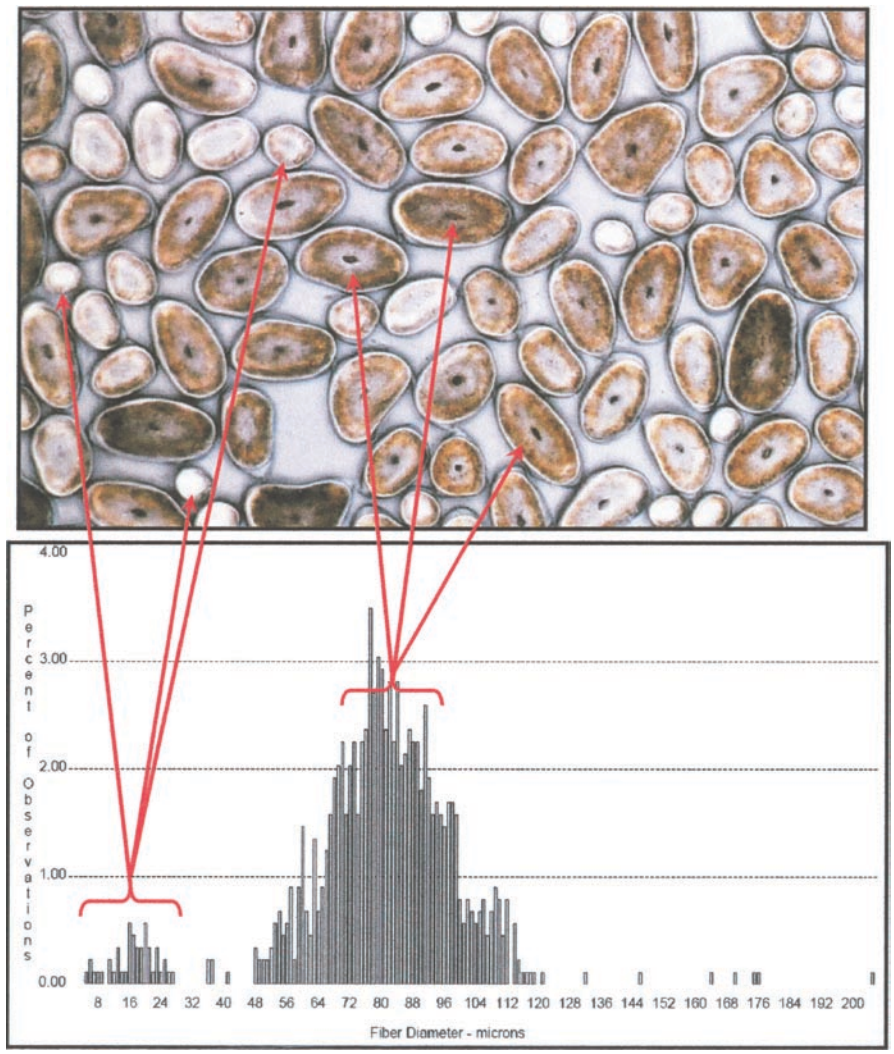

Fig. 6.3 Example of OFDA modes and cross-sectional area diversity

images were taken 24–72 h apart as described above. The study was approved by the local Institutional Review Board at Case Western Reserve University in Cleveland, OH. Analysis of variance was performed by scalp site on hair growth rate, hair count, percent anagen, anagen to telogen ratio, and hair diameter with menopausal status as the factor. In addition, mixed model analysis was performed by menopausal status on all hair growth and diameter parameters with scalp site as the factor and subject as random in the model. Furthermore, the relationships between each hair growth and diameter parameter and menopausal status and age were displayed using scatter plots.

In the additional study, hair samples were collected from the frontal scalp in 350 subjects between 18 and 84 years. The study was conducted at CRG, Inc., Minneapolis MN under the supervision of an Institutional Review Board. The mean diameter was compared across the three groups using analysis of variance. In all groups 100–200 hairs were collected per scalp site.

6.3 Results

6.3.1 Demographics

In the initial study, a total of 44 women were enrolled; 20 of them postmenopausal. The age range was 22–84. Sixty four percent of the women identified as Caucasian and 27% as African American. Of the Caucasian women, 12 were premenopausal and 16 were postmenopausal. There were more African American women who were premenopausal ($N = 9$) as compared to postmenopausal ($N = 3$). In situations where the technical quality of the captured image was deemed poor for analysis, the data were excluded.

In the larger follow-up study 177 subjects were between 40 and 60 years old with 54 premenopausal subjects, 33 perimenopausal subjects, (perimenopause was defined as irregular periods or cessation of periods for less than 12 months), and 90 postmenopausal subjects.

6.3.2 Hair Growth Rate

In all women ($N = 33$ for frontal, $N = 34$ for occipital), the hair growth rate was significantly lower in frontal than occipital scalp ($p = 0.006$). The growth rate for premenopausal women was significantly higher than postmenopausal women at both the frontal and the occipital sites ($p \leq 0.074$) (Fig. 6.4a). A scatter plot of hair growth rate and age showed that hair growth rate in the frontal scalp first increases and then decreases with age with the maximum hair growth rate occurring around the early 40s (Fig. 6.4b). In the occipital scalp site, the hair growth rate tends to decrease only slightly with age (Fig. 6.4c).

6.3.3 Hair Density

In both premenopausal and postmenopausal women ($N = 33$ for frontal, $N = 34$ for occipital), the frontal scalp had significantly higher hair counts than the occipital scalp ($p \leq 0.04$). When comparing the two groups of women, postmenopausal women had significantly lower frontal hair counts than premenopausal women ($p = 0.091$) (Fig. 6.5a). Interestingly, menopausal status did not affect hair density in occipital scalp, indicating differential effects of hormonal status on frontal vs. occipital scalp. In this study, both frontal and occipital hair density showed a wide scatter of values but showed an increasing trend with age before 40 years and a decreasing trend with age after 40 years (Data not shown). Other data, both published and unpublished, show a slow, but steady, decrease in hair density with age. One unpublished study (authors' communication) clearly shows this trend on frontal scalp (Fig. 6.5b).

6.3.4 Percent Anagen Hairs

Regardless of scalp site, premenopausal women had higher % anagen hairs than the postmenopausal women (frontal Site $p = 0.055$, occipital Site $p = 0.004$) (Fig. 6.6a). Scatter plot analysis of the frontal scalp showed a peak in the percent anagen hairs near age 40 with a decline thereafter. There was no correlation of age and percent anagen hair in the occipital scalp, further indicating the differential regulation of frontal and occipital scalp by hormonal status (Figs. 6.6b, c).

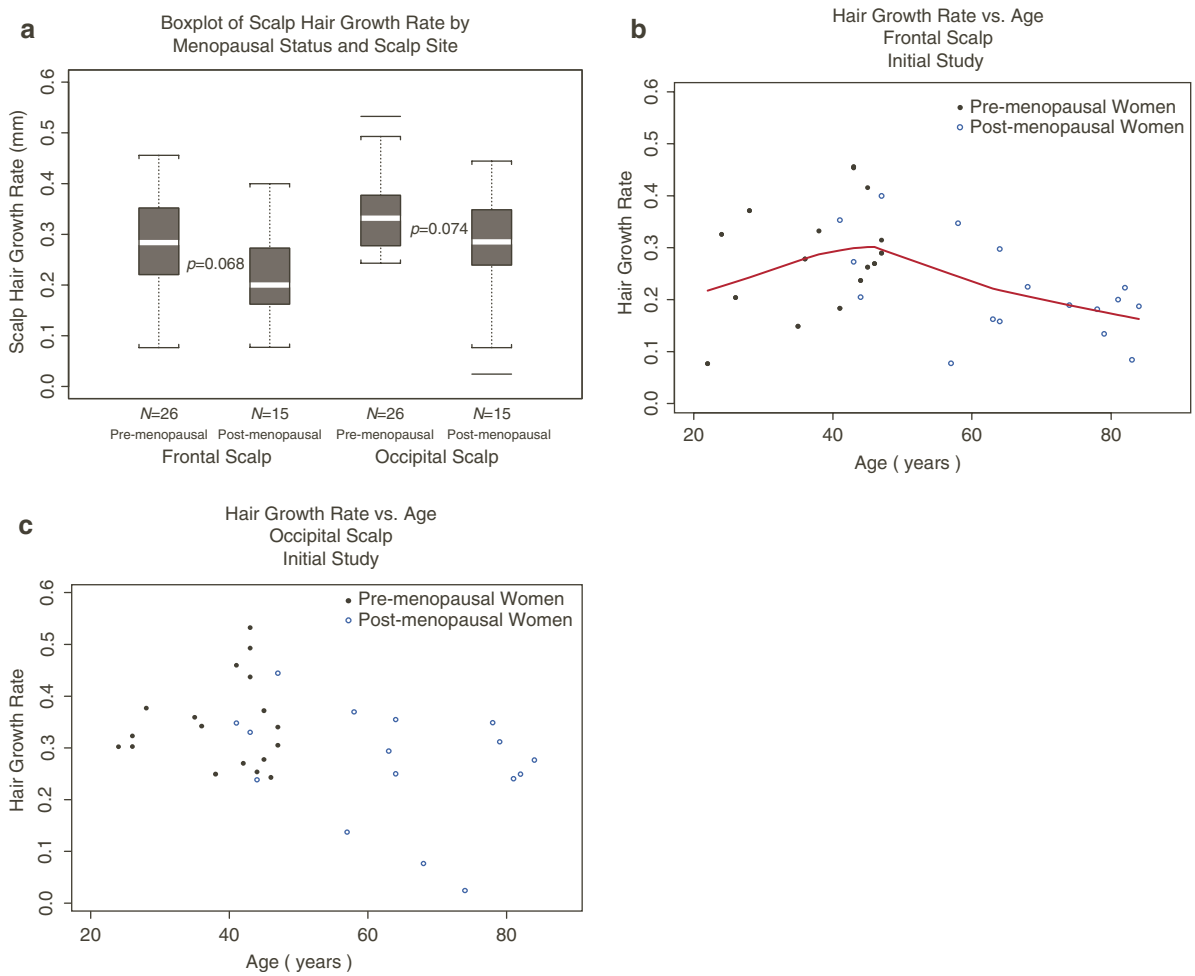

Fig. 6.4 (**a**) Growth rate in pre- and postmenopausal states. (**b**) Growth rate vs. age on frontal scalp. (**c**) Growth rate vs. age on occipital scalp

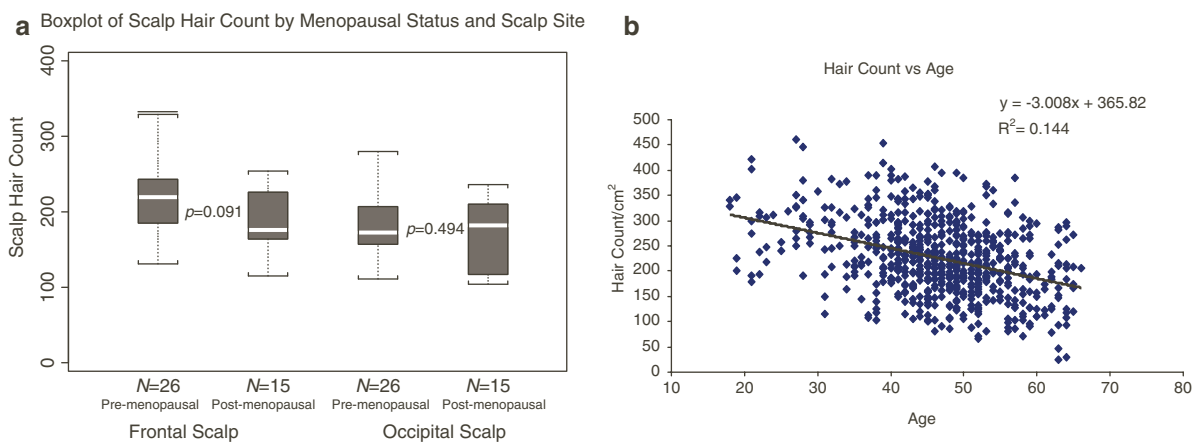

Fig. 6.5 (**a**) Hair count by menopausal status. (**b**) Hair count by age (independent study, unpublished) (similar to other data, $N = 1{,}422$, all frontal)

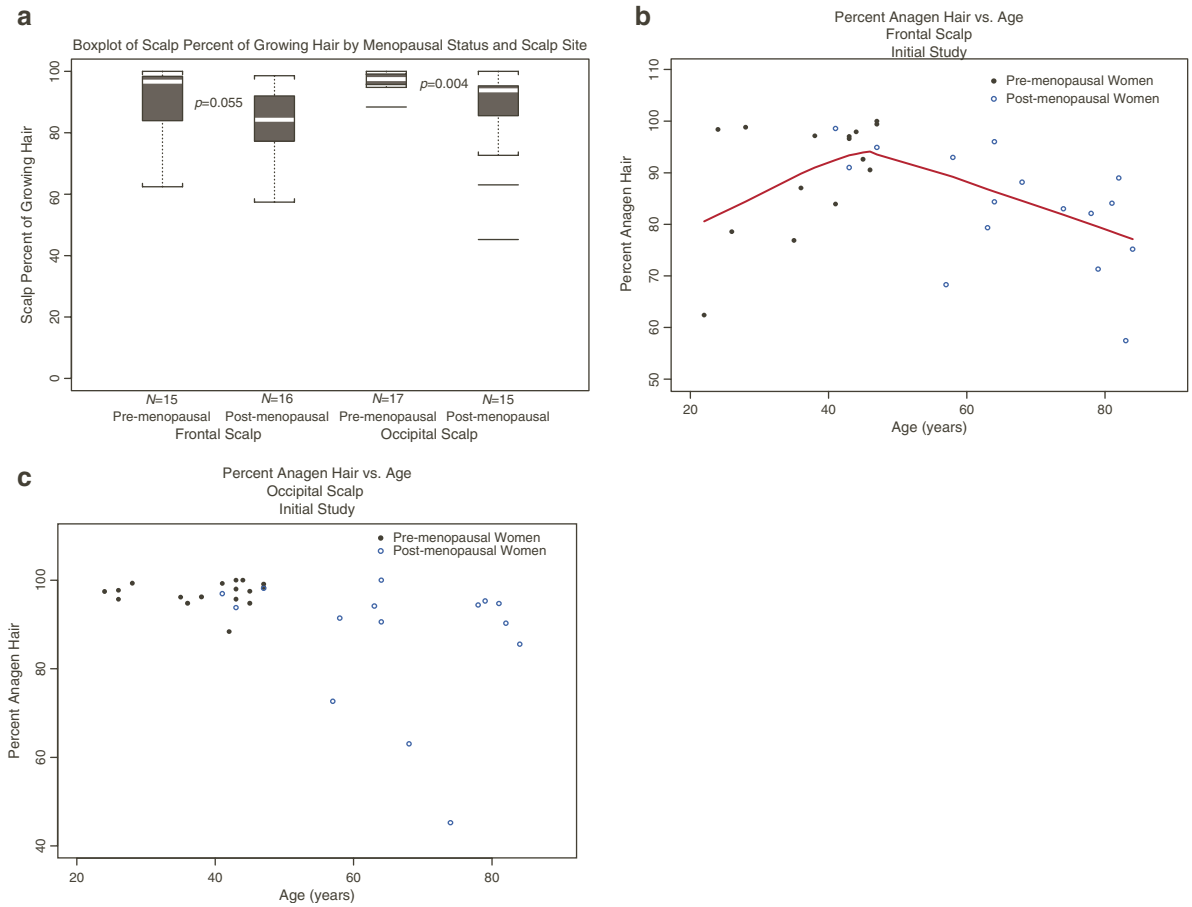

Fig. 6.6 (**a**) Percent growing hairs by menopausal status. (**b**) Percent growing hairs by age, frontal scalp. (**c**) Percent growing hairs by age, occipital scalp

6.3.5 Hair Diameters

In parallel with the previous measures, mean hair diameters were significantly higher in premenopausal compared to postmenopausal women for the frontal site ($p = 0.067$), but there was no change in the occipital hair diameter ($p = 0.124$) (Fig. 6.7a). Scatter plots also showed a trend to lower hair diameter with age on the frontal, but not on the occipital scalp (Fig. 6.7b, c).

However, when looking broadly at the distribution of individual hair diameters, the distribution of hair diameters clearly differed in these two groups for subjects aged between 40 and 60 years (Fig. 6.8a, b). The distribution of hair diameters was not normal and showed 2 peaks with the majority of hairs in the "fine to coarse" distribution of 25–150 μm. Premenopausal women had more coarse hairs (>100 μm) as compared to postmenopausal women. No clear correlation was found between hair density and hair diameters.

In further work, we undertook an expanded study of hair diameter in women of age 18 years or older (on the frontal site only) in order to more definitively determine if there was a relationship between hair diameter and menopausal status independent of age (see subjects and methods). Again, in the larger study, mean hair diameters for the frontal scalp were significantly higher in premenopausal compared to the postmenopausal and perimenopausal women (data not shown). No significant difference in mean hair diameter was found between peri- and postmenopausal women

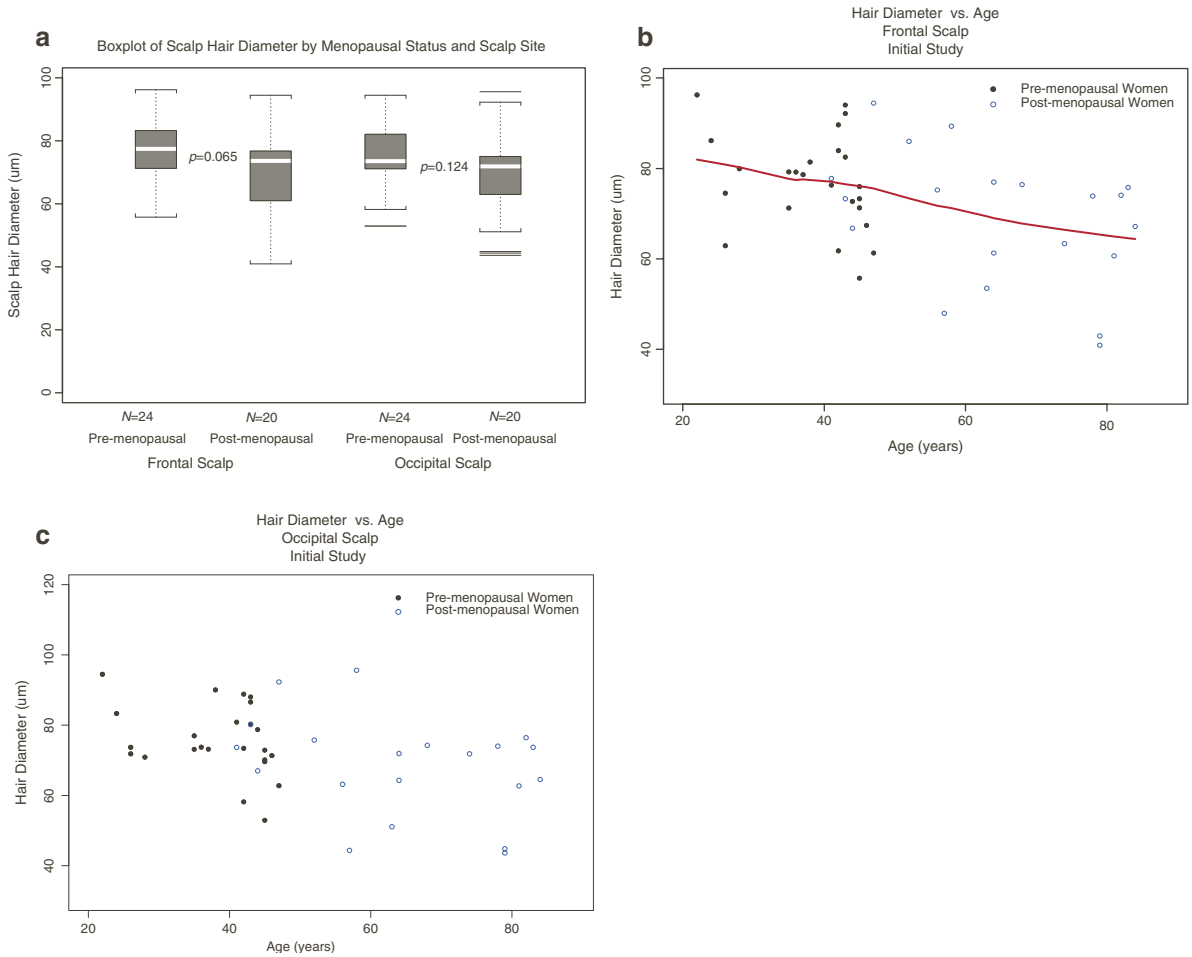

Fig. 6.7 (**a**) Scalp hair diameter by menopausal status. (**b**) Scalp hair diameter by age, frontal scalp. (**c**) Scalp hair diameter by age, occipital scalp

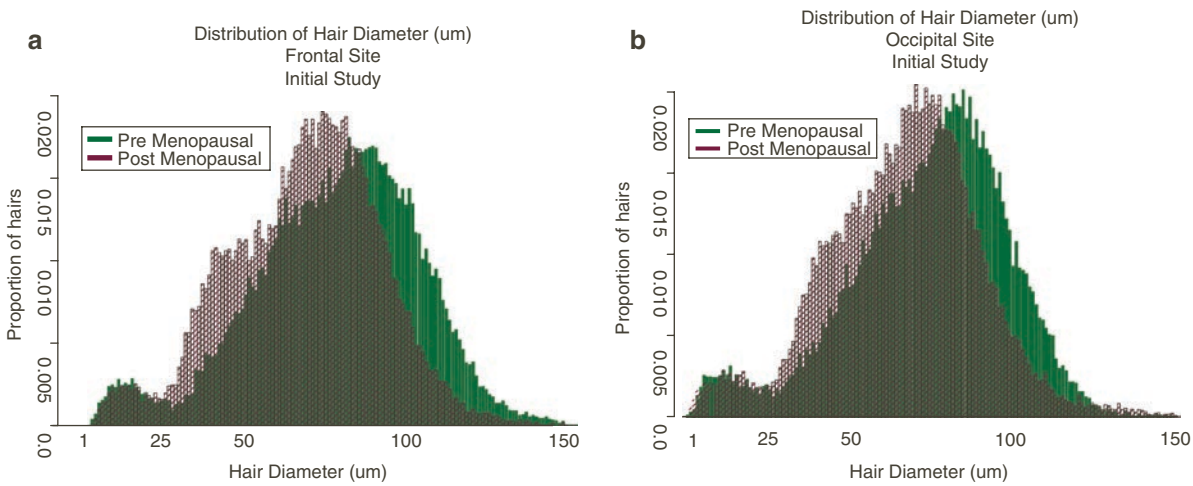

Fig. 6.8 (**a**) Diameter distribution on frontal scalp. (**b**) Diameter distribution on occipital scalp

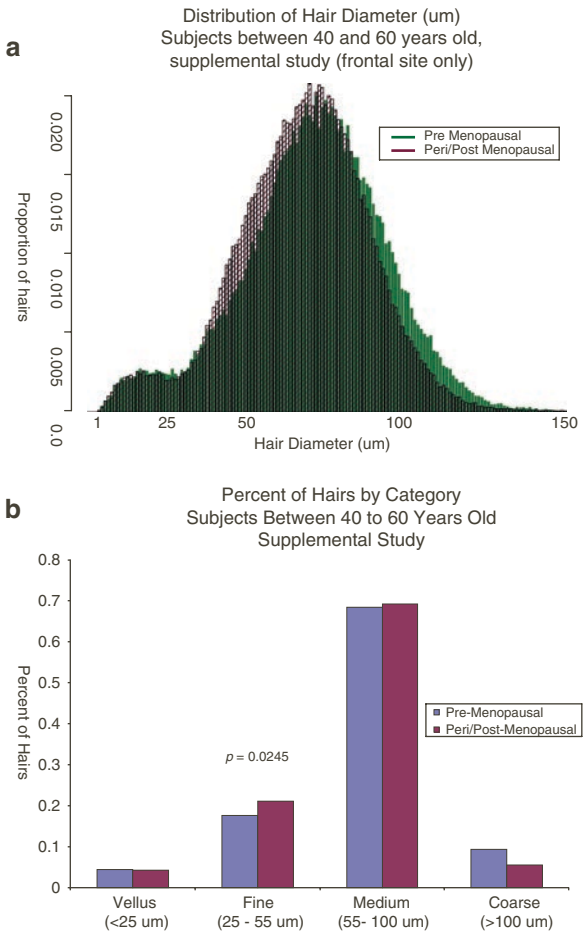

Fig. 6.9 (**a**) Scalp hair diameter distribution by menopausal status, frontal site. (**b**) Scalp hair diameter distribution by age, fine, medium, and coarse

(p = 0.6). As a result, the peri- and postmenopausal groups were combined for a broader final analysis. Inclusion of the additional subjects allowed differentiation of the effect of menopausal status independent of age. The distribution of hair diameters did differ between premenopausal and post/perimenopausal women, independent of age, supporting the role of hormonal status in decreasing hair diameter (Fig. 6.9a).

Further analysis revealed that the hair diameter in the larger cohort also followed a bimodal distribution with majority of the hairs in the "fine to coarse" distribution of 25–150 μm. Premenopausal women had significantly more coarse hairs (>100 μm) and significantly fewer fine hairs (25–55 μm) as compared to peri/postmenopausal women (p = 0.0245). The medium (55–100 μm) and vellus (<25 μm) hairs did not differ between these two groups (Fig. 6.9b).

6.4 Discussion

In women without overt hair loss there are significant differences in hair growth characteristics between pre- and postmenopausal women, specifically hair growth rate, density, percent anagen, hair diameters and hair diameter distributions. This is more pronounced in the frontal compared to the occipital scalp. It is known from embryologic and patterning studies that the origin of the frontal and occipital scalp is different and that follicles from these two areas respond differently to hormonal influences [30]. Prior studies of hair characteristics in the frontal scalp of women have shown similar findings [3, 34]. In a British study, 424 women aged 18–99 years were examined and a photographic method used to measure hair density; hair diameters were also evaluated [3]. It was determined that hair densities decreased with age whereas hair diameters peaked at around age 30 and subsequently declined. In another study of 159 Japanese women aged 14–68, hair parameters were measured using a phototrichogram [34]. In this study, hair densities also decreased with age; the ratio and growth rate of anagen as well as mean hair diameter declined after the 40s.

To our knowledge, menopausal status has not been previously evaluated as an independent variable affecting hair characteristics. In these studies the hair characteristics of growth rate, density, percent anagen, overall diameter and diameter distribution were compared on frontal and occipital scalp and in pre- and postmenopausal women. Where data were available, comparisons were also made for parameters vs. age.

Changes in hair measures are accentuated in the frontal scalp compared to the occipital scalp in postmenopausal women. Frontal scalp was significantly different in pre- vs. postmenopausal women in each of the four parameters measures, but occipital scalp varied by menopausal status only for growth rate and percent anagen. Based on the scatter plot analysis, age correlations were noted with all the measured hair characteristics *except for* occipital growth rate, percent anagen and hair diameter. One interpretation of these findings is that diffuse hair changes that are a result of a decrease in hair growth (rate) and alterations in hair cycling (percent anagen) may be specific changes that result from menopause. It is intriguing whether the occipital scalp follicles may be differentially regulated with respect to as yet unidentified signals during menopause. These findings are in keeping with other studies and clinical observations that suggest estrogen plays an important role in the follicular cycle.

In all women studied, there was a higher hair density on the frontal scalp than the occipital scalp. The findings corroborate the patients' history of no hair loss. An additional finding was that frontal and occipital hair density showed a wide range of values but showed a decreasing trend after age after 40. As noted above, prior studies of women without hair loss have also shown a wide, but normal distribution of scalp hair densities that steadily declines with age [3, 34]. Therefore, it is likely that chronologic age, but not menopausal status dictates alterations in hair density.

OFDA analyses of mean hair diameter and hair diameter distribution showed that mean hair diameter was significantly lower in the postmenopausal women relative to premenopausal. In the larger, frontal scalp only study, hair diameter was shown to increase until the age of 40 years, and then decrease after age 40. However, detailed analysis of the larger cohort revealed that menopause was well-correlated with a significant drop in frontal hair diameter independent of age. Further, hair diameter distributions were different between pre- and postmenopausal women. Specifically, hair diameter distribution curves showed a change or "downshift" in the larger diameter hair fibers in peri/postmenopausal women, while small diameter hairs remained essentially unchanged.

Although traditional techniques of hair diameter measurement such as light microscopic micrometry and phototrichogram measurements can also provide accurate measurements of hair diameter, we were able to further refine and expand on this hair parameter using OFDA. Since hair diameters do not show a normal distribution, prior reports of mean hair diameters may be a misleading measurement especially when comparing two groups or when assessing changes over time [3]. Instead, there are frequently two and sometimes three "modes" or "peaks" in the distribution curve of hair diameters. Further compounding the difficulty in measuring this hair parameter is the need to account for major and minor axes. Large volume and reliable measurements of hair diameters using OFDA allows for an accurate and visually dramatic reporting of hair diameter distributions. Thus, this new technology allows for an entirely new approach to this hair parameter. The "downshift" in large diameter hairs seen when comparing the pre- and peri/postmenopausal groups suggests that larger diameter hairs may be more "susceptible" to the hormonal changes that occur during menopause. A similar diminution in larger diameter hairs is seen in androgen-dependent hair alopecia in men and women. Considering the fact that androgen receptors and estrogen receptors are both present in the dermal papilla of the hair follicle, it is conceivable that both of these receptors are involved in modulation of the hair size.

One prior study assessing growth rate of pigmented vs. white hairs in postmenopausal women showed a significantly slower growth rate and thickness, mainly of the pigmented hairs, the biologic significance of this finding is as yet unclear [38]. In our study the pigmented and white hairs were not studied as a separate variable; however efforts were made to maximize efficiency of measurement of white hairs by use of a hair dye.

Although this study does not explore the possible pathogenetic causes for the differences observed in pre- and postmenopausal women, the known effects of estrogen on the hair make this hormone the leading target for ongoing study. Until the complex molecular effects of estrogen on peripheral tissues are fully understood, progress can be made in identifying and describing relevant clinical observations. Further study of hair changes in response to menopause provides an important opportunity for identification of treatments targets and strategies that may significantly benefit women.

Take-Home Pearls

- Menopausal status may influence hair parameters, specifically hair growth rate and number of hairs in anagen.
- Changes in hair measures are accentuated in the frontal scalp compared to the occipital scalp in postmenopausal women.
- Scalp hair density decreases with age, and does not seem to be significantly influenced by menopausal status.
- Hair diameter is lower in the frontal scalp in postmenopausal women; this finding is independent of age.
- Hair diameters are not normally distributed, so diameter distribution as opposed to mean diameter gives a more accurate assessment. Using this new measurement technology we see a decrease in high diameter hairs in postmenopausal women.

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7 Comparative Histopathology of Androgenetic Alopecia, Diffuse Alopecia, and Senescent Alopecia

David A. Whiting

Core Message

Diagnosis of senescent alopecia

- Onset after 60 years of age
- Possible family history of balding
- Diffuse thinning over the scalp
- Reduced hair number and diameter
- Miniaturized hairs frequent, but not invariable
- Inflammatory changes not always significant

7.1 Introduction

The concept of senescent alopecia evolved from clinical observations that diffuse hair thinning involving the whole scalp may develop after the age of 50 years in people with no family history of balding. Scalp surface studies favored the idea that hair follicle density, anagen to telogen ratios, and hair shaft diameters decrease steadily with advancing age [1–3]. Originally these conclusions were based on hair counts which were not confirmed by histological evaluation. Hair miniaturization was not thought to occur. Histological evidence favoring this concept was presented in 1988 by Kligman, although he did state that senescent alopecia could be superimposed on androgenetic alopecia [4]. However, a recent study has shown that follicular miniaturization does occur in patients diagnosed as senescent alopecia [5]. Originally, the roles of androgens and genetic factors in patients with senescent alopecia were not well-clarified, but now new data on patterns of androgen metabolism and gene expression in senescent and androgenetic alopecia have been generated by Northern blot [5] and microarray analysis [6]. These findings are presented by Paradi Mirmirani in Chap. 8.

7.2 Histopathology

The three common clinical diagnoses of nonscarring, diffuse thinning in patients over the age of 50 years are pattern baldness, diffuse alopecia, and senescent alopecia. In senescent alopecia the histopathology is controversial but it is more specific in androgenetic alopecia [7] and diffuse alopecia [8]. Sperling [9] has pointed out that many patients with senescent alopecia show evidence of mild, concomitant, androgenetic alopecia; he reported a slight decrease in the total number of hairs with less than 15% telogen hairs, a terminal:vellus ratio of at least 2:1, and an absence of deep perifollicular inflammation. It has been noted that inflammation and fibrosis are more common around the upper follicle in androgenetic alopecia than in diffuse alopecia and controls [4, 8, 10, 11]. In the pilot study by Price et al., 11 males with scalp thinning after the age of 60 years were compared with 4 age-matched controls and younger men with typical androgenetic alopecia [5]. Biopsies were taken from frontal and occipital scalp. With routine staining senescent alopecia was found to be indistinguishable from androgenetic alopecia histologically in both biopsy sites, primarily reflecting follicular miniaturization. Inflammatory changes were not significant.

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In the Hair and Skin Research and Treatment Center (HSRTC) at Baylor, the term senescent alopecia has not been used as a primary diagnosis. Thus all possible examples of this condition in patients aged over 50 years have been included in the diagnosis of pattern alopecia or diffuse alopecia. A review of the 21-year experience at the HSRTC with biopsies from all age groups with diffuse hair thinning has revealed two major histopathologic patterns. One shows significant hair miniaturization with a terminal:vellus ratio of less than 4:1 compatible with male- or female-pattern baldness (Figs. 7.1 and 7.2) and the other a normal terminal:vellus ratio greater than 4:1 compatible with diffuse alopecia from acute or chronic telogen effluvium or other causes (Figs. 7.3 and 7.4). Vellus hair diameter is defined as 0.03 mm or less and terminal hair diameter as greater than 0.03 mm.

An age-related analysis of follicular counts in horizontal sections of 2,127 4-mm punch biopsies from the scalp crown in pattern and diffuse alopecia has some relevance here. The 22 normal controls are insufficient for age analysis by decade, but the 852 biopsies of diffuse alopecia can serve as controls for this purpose. These findings are compared with those in 254 biopsies of male pattern and 1,021 biopsies of female pattern alopecia. It can be seen in Table 7.1 that the mean follicular counts in controls and diffuse alopecia are compatible. The only minor differences are a slight decrease in total hairs and a relative increase in terminal telogen hairs (TT) in diffuse alopecia. It therefore seems reasonable to assume that the terminal and vellus hair

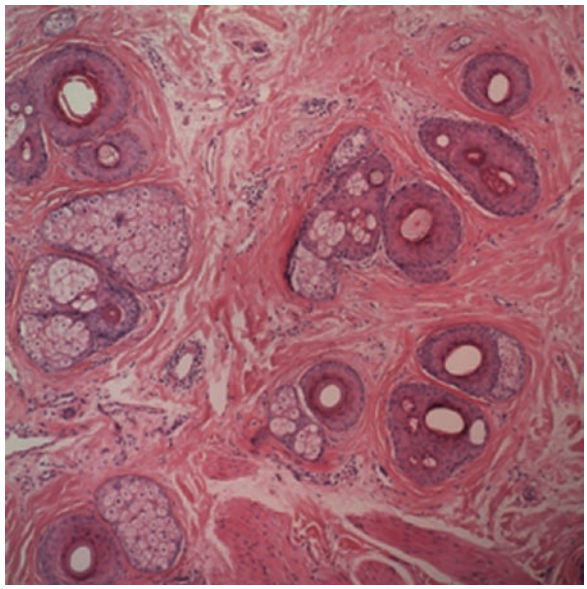

Fig. 7.2 Senescent alopecia histologically compatible with androgenetic alopecia. Horizontal section: terminal: vellus ratio 1.8:1. Mild inflammatory infiltrate (original magnification X100, H and E)

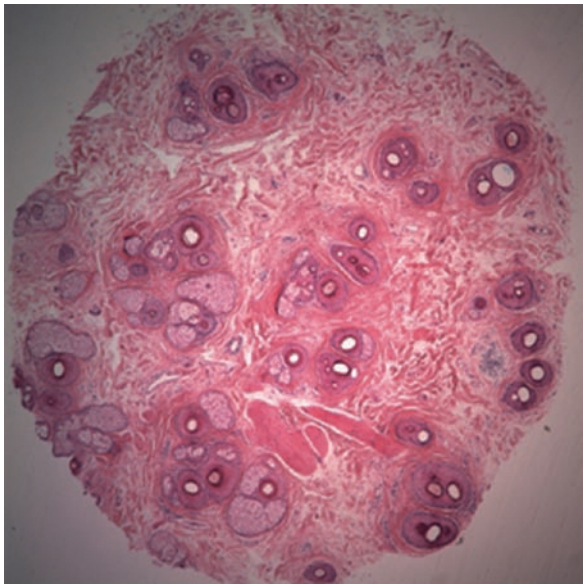

Fig. 7.1 Senescent alopecia histologically compatible with androgenetic alopecia. Horizontal section: terminal: vellus ratio approximately 1.8:1. Mild inflammatory infiltrate (original magnification X 40, H and E)

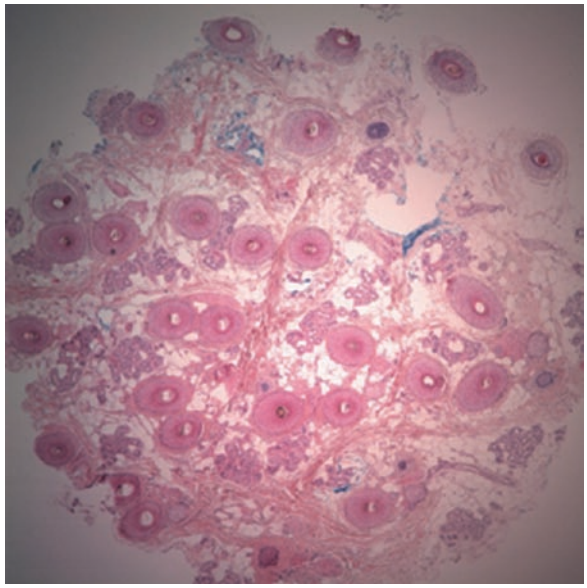

Fig. 7.3 Senescent alopecia histologically compatible with diffuse alopecia. Horizontal section: terminal: vellus ratio 9.7:1. (1 vellus hair bulb and 2 vellus telogen hairs). No significant inflammation (original magnification X 40, H and E)

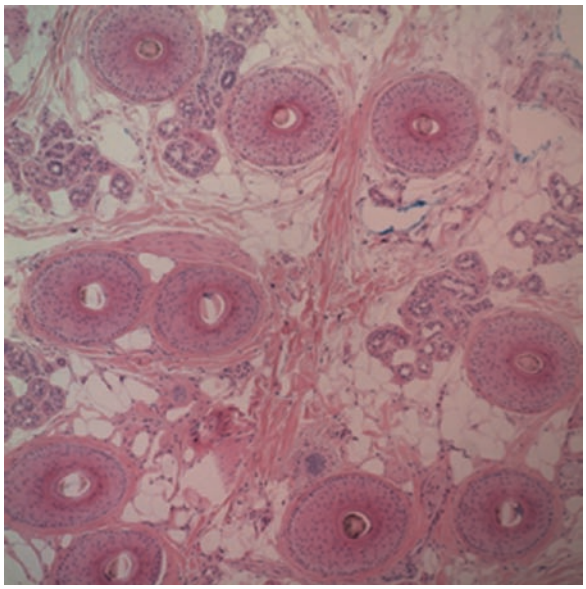

Fig. 7.4 Senescent alopecia histologically compatible with diffuse alopecia. Horizontal section: terminal: vellus ratio 10:1. No significant inflammation (original magnification X100, H and E)

counts by decade in diffuse alopecia approximate to normal hair counts, in contrast to the findings in pattern baldness.

In 852 (33 females:1 male) cases of diffuse alopecia, the total number of terminal and vellus hairs (VH) remained steady at an average of 39.12, a hair density of 3.1/mm^2 (310/cm^2), until age 49 (Table 7.1). This number correlates well with normal controls. There was only a 5.7% reduction in the average follicular count to 36.89, a hair density of 2.9/mm^2, over the next 30 years until age 79. There was no significant increase in VH during this period. A significant drop in total follicular counts of 22% compared to baseline, resulting in a hair density of 2.4/mm^2, was seen in the 17 patients in their 80s and 90s. The majority of the patients biopsied in the diffuse alopecia group carried the diagnosis of chronic telogen effluvium and did not progress to severe baldness. It seems possible that the diagnosis of senescent alopecia is appropriate in this group in patients with a follicular density of 2.4/mm^2 or less, or aged 80 or more.

In 254 cases of male-pattern androgenetic alopecia the total number of terminal and VH in a 4 mm punch biopsy cut horizontally, remained steady at an average of 35.56, a hair density of 2.8/mm^2, until age 59 (Table 7.2). There was a significant increase in VH during this period. A significant drop in total follicular counts of 18% compared to baseline, resulting in a hair density of 2.3/mm^2, was seen in the ten patients in their sixties to nineties, in whom senescent changes could be considered.

In 1,021 cases of female pattern alopecia, the total number of terminal and VH in a 4 mm punch biopsy

Table 7.1 *Diffuse alopecia:* follicular counts in horizontal sections of 4 mm scalp biopsies at hair and skin research and treatment center (HSRTC) 1987–2008

Age years	Number of Pt's	TA	TT	TH	VH	Total H	FU	FS	T:V	TA%	TT%
10–19	22	31.5	3.3	34.8	4.8	39.6	13	2.9	7.3:1	91	9
20–29	75	31.3	3.2	34.5	4.7	39.2	13.1	2.6	7.3:1	88	12
30–39	172	31.5	3.4	34.9	5	39.9	13.7	2.9	7.0:1	90	10
40–49	252	30.9	3.2	34.1	4.4	38.5	13.3	2.7	7.8:1	89	11
50–59	163	28.3	3.4	31.7	4.1	35.8	12.6	3.3	7.7:1	89	11
60–69	108	27.8	4.1	31.9	3.9	35.8	13.3	3.5	8.1	87	13
70–79	43	27	3.9	30.9	3.6	34.5	13.7	4.2	8.5:1	87	13
80–89	14	23.4	3.4	26.8	4.1	30.9	12.7	3.6	6.5:1	87	13
90–99	3	22.6	3.7	26.3	2.3	28.7	12.3	1.7	11.3:1	92	8
Total 10–99	852	29.8	3.4	33.2	4.4	37.6	13.2	3	7.6:1	89.7	10.3
Controls	22			35	5	40	13	1–2	7:1	93.5	6.5
Mean age 43											

TA terminal anagen hairs; *TT* terminal telogen hairs; *TH* terminal anagen and telogen hairs; *VH* vellus hairs; *Total H* total terminal and vellus hairs; *FU* follicular units; *FS* follicular stellae; *T:V* terminal to vellus hair ratio

Table 7.2 *Male-pattern alopecia* follicular counts in horizontal sections of 4 mm scalp biopsies at HSRTC 1987–2008

Age years	Number of pt's	TA	TT	TH	VH	H-Total	FU	FS	T:V	TA%	TT%
10–19	22	16.3	3.9	20.1	11.5	31.6	12.6	4.6	1.7:1	81	19
20–29	52	20.5	4.7	25.2	13.3	38.5	13.3	6.4	1.9:1	81	19
30–39	97	17.7	4.4	22.1	13.9	36	13.1	8.7	1.6:1	80	20
40–49	56	15.5	4	19.5	15.3	34.7	13.6	10.4	1.3:1	79	21
50–59	17	13.9	3.4	17.3	14.7	32	12.6	6	1.2:1	80	20
60–69	4	7	3.3	10.3	19	29.3	13.3	8.8	0.5:1	68	32
70–79	4	18	4.8	22.8	6.3	29.1	11	5.3	3.6:1	79	21
80–89	0	0	0	0	0	0	0	0	0	0	0
90–99	2	12	2.5	14.5	14.5	29	14	10.5	1:1	83	17
Total 10–99	254	16.6	4.2	20.8	14.6	35.4	13.2	8.2	1.4:1	80.0	20
Controls	13			36	6	42	13	2	6:1	94.0	6.0
Mean age 39											

TA terminal anagen hairs; *TT* terminal telogen hairs; *TH* terminal anagen and telogen hairs; *VH* vellus hairs; Total H total terminal and vellus hairs; *FU* follicular units; *FS* follicular stellae; *T:V* terminal to vellus hair ratio

cut horizontally, remained fairly steady at an average of 33.41, a hair density of 2.7/mm^2, until age 59 (Table 7.3). There was a significant increase in VH during this period. A definite drop in total follicular counts of 11.2% compared to baseline, resulting in a hair density of 2.3/mm^2, was seen in the 171 patients in their sixties to nineties, a number of whom may have been complicated by senescence.

7.3 Discussion

The diagnosis of senescent alopecia as a single entity is complicated by overlapping cases of androgenetic or diffuse alopecia. The issue is further confused by the fact that the balding process can extend well down into the occipital and parietal scalp in some patients with severe pattern alopecia, especially females. Indeed,

Table 7.3 *Female-pattern alopecia*: follicular counts in horizontal sections of 4 mm scalp biopsies at HSRTC 1987–2008

Age years	Number of pt's	TA	TT	TH	VH	H-Total	FU	FS	T:V	TA%	TT%
10–19	37	19.6	3.1	22.7	12.5	35.2	13.3	6.4	2.4:1	86	14
20–29	125	19.9	3.2	23.1	11.2	34.2	13.5	7.1	2.4:1	86	14
30–39	220	19.8	3.2	23	11.9	34.8	13.3	5.8	2.4:1	86	14
40–49	234	18.8	3.3	22	10.7	32.8	13	5.8	2.4:1	85	15
50–59	234	18.4	3.1	21.4	10.6	32	13	6.3	2.5:1	86	14
60–69	106	16.4	2.8	19.2	11.3	30.5	12.7	6.3	2.3:1	85	15
70–79	48	15.7	2.5	18.2	9.7	27.9	12.4	6.3	2.3:1	86	14
80–89	7	18.7	2.6	21.3	9.7	31	14.4	5.3	2.5:1	88	12
90–99	10	17	2.6	19.6	8.7	28.3	12.1	3.6	3.0:1	87	13
Total 10–99	1,021	18.5	3.1	21.6	11.1	32.7	13.1	5.6	1.9:1	86.00	14
Controls	9			34	3	37	13	1	11:1%	93.50	6.50
Mean age 48.5											

TA terminal anagen hairs; *TT* terminal telogen hairs; *TH* terminal anagen and telogen hairs; VH vellus hairs; *Total H* total terminal and vellus hairs; *FU* follicular units; *FS* follicular stellae; *T:V* terminal to vellus hair ratio

such patients are not suitable for hair transplantation due to the large reduction of hairs in the donor area. It can also be noted from the biopsy tables that while most men with male-pattern alopecia present by age 49, there are some exceptions. In contrast, patients with female pattern alopecia or diffuse alopecia commonly present for a further 20 years up to at least age 69. Thus both diffuse and pattern alopecia can involve scalp hair extensively up to an age of 70 and later.

The original concept of senescent alopecia has been modified by an increased age of onset of at least 60 years and the presence of follicular miniaturization with changes in patterns of gene expression [5, 6]. Furthermore, a family history of balding may be present. However, these findings are based on a small series of patients and should be further tested. Presumably, the patients were selected for the study by their clinical presentation. Based on the additional biopsy data from the two histopathologic patterns in older patients presented here, it might be rewarding to focus on all patients with diffuse thinning presenting over the age of 70. No doubt many will have histology compatible with androgenetic alopecia but others may show the histology of a diffuse alopecia. In future studies, it would be helpful if the same histologic and immunostains [5] and microarrays [6] were used on biopsies from these patients. This is important since senescent changes can be superimposed on both androgenetic and diffuse alopecia, or on an otherwise normal scalp.

Take Home Pearls

- Senescent alopecia may be superimposed on androgenetic or diffuse alopecia or normal scalp.
- Biopsy all patients with presumed senescent alopecia.
- Blood tests should include at least a complete blood count, comprehensive metabolic panel, thyroid panel and iron studies.
- Treatment should be appropriate for either androgenetic or diffuse alopecia, depending on the presence or absence of miniaturized hairs.
- Topical minoxidil and/or oral finasteride may be helpful.

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8 Comparative Gene Expression Profiling of Senescent and Androgenetic Alopecia Using Microarray Analysis

Paradi Mirmirani and Pratima Karnik

Core Messages

- Androgenetic (AGA) and senescent alopecia (SA) are common nonscarring hair loss disorders. The histopathology of both disorders involves follicular downsizing, and yet, they differ in the age of onset, pattern of hair loss, and hormonal involvement.
- We compared gene expression profiles of scalp biopsies from age-matched men with AGA, SA, and normal controls.
- Our data suggest that follicular downsizing in AGA is associated with altered expression of genes required for hair follicle cycling.
- In stark contrast, the transcriptional profile of SA reveals mitochondrial dysfunction and oxidative stress response, which are characteristic of aging tissues.
- These marked differences between AGA and SA suggest that they are two distinct disorders that present diverse mechanisms for a final common phenotype of follicular downsizing.

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8.1 Introduction

It has long been observed that human hair is prone to thinning with advancing age and such hair thinning is often identified as a marker of senescence in humans and other mammals. Genetic disorders of premature senescence such as progeria indeed show a phenotype of hair loss [28]. The clinical criteria for diagnosing senescent alopecia (SA) include: (1) The absence of a family history for male and female androgenetic alopecia (AGA) and (2) Hair thinning that does not become apparent until after approximately 50 years of age [23,27, 57]. However, the cause of this senescent or age-related thinning has been poorly characterized. Even the name "senescent alopecia" (SA) has been slow to gain acceptance since many clinicians consider this type of hair loss to be a continuum of AGA. Others take issue with the term "senescent" since there has been little evidence that aging signals are directly involved in the etiology of hair loss in the elderly and instead refer to "early onset" and "late onset" patterned baldness or thinning. Perhaps, the controversy in nomenclature stems from conflicting clinical and histopathologic descriptions of SA in the literature. Clinically, the hair thinning in SA is often described as being diffuse when seen in its "pure form" but having both a diffuse and patterned thinning when seen in combination with AGA [57, 58], whereas others consider SA to have a pattern similar to AGA but without full balding [23]. Measurements of scalp hair characteristics have clearly shown decreased density, diameter, and hair cycle with advancing age [2, 6, 9, 62]. However, the histopathologic descriptions of SA are equally diverse as the nomenclature and clinical descriptions. SA is reported by some to be similar to normal scalp but with mildly

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decreased density and no miniaturization [27, 57, 58, 73], whereas others consider it to be identical to AGA with follicular downsizing [50]. Since SA likely coexists with AGA in many patients, it seems essential to better delineate and differentiate the two in order to advance our understanding of SA.

8.1.1 Differences Between AGA and SA

The differences between AGA and SA are summarized in Table 8.1. Unlike SA, early onset AGA typically begins in the teens, twenties, and thirties in a patterned distribution in both men and women [47], and its pathophysiology has been extensively studied and well characterized [8, 36]. In AGA, there is a gradual transformation of large terminal hair follicles to miniaturized ones under the influence of circulating androgens that produce smaller and finer hairs with a shorter anagen cycle [8, 18, 36, 47]. This transformation, which can be seen as early as the prepubescent years, occurs only in certain regions of the scalp [48]. In men, the scalp regions affected by the miniaturization process are the frontal hairline, top, and vertex scalp; the temporo-occipital region is largely unaffected even in those with extensive balding [42]. Hair transplantation further demonstrates that hair follicles from the occipital scalp are affected very little by androgen-mediated hair miniaturization [44]. After transplantation to the frontal scalp, the occipital hairs continue to grow proving that androgen responsiveness is genetically determined at the level of the follicle. However, it has been observed that in men with early onset and advanced AGA, further hair thinning can occur in the "androgen insensitive" occipital area with advancing age. This observation suggests that SA, in contrast to AGA is not limited to a specific scalp region and may not be hormonally mediated.

It has been clearly established that the pathophysiology of early onset AGA is hormonal in nature. In younger men and women with AGA, there is a substantial increase in the local transformation of testosterone to dihydrotestosterone (DHT) in the frontal anagen hair follicles by the enzyme 5α-reductase [52]. The important role of DHT has been demonstrated in patients with male pseudohermaphroditism due to congenital deficiency of 5α-reductase type II who do not develop temporal recession [17]. DHT, which has a five times higher affinity for the androgen receptor (AR) compared to testosterone triggers specific gene sites responsible for the gradual miniaturization of genetically programed hair follicles [35]. Studies evaluating the correlation between serum androgen levels with degree of hair loss have been controversial, but it may be that an elevated ratio of DHT/T is significant in men with severe AGA [21]. It has been suggested that the various clinical patterns of AGA in men and women may reflect quantitative differences in levels of AR and steroid-converting enzymes in specific scalp regions at different ages. However, since there may be other nonandrogen causes of hair thinning in women which have no counterpart in men, the term female pattern hair loss (FPHL) has been coined to encompass the clinical phenotype of hair loss in the central scalp region [40, 41]. It may be that gender-specific hair growth differences may occur due to the modulatory role of estrogen receptor beta, which has only recently been identified [64, 65]. Although the effect of estrogens in follicular controls is less well understood than androgens, a variety of clinical data suggest it is an attractive candidate for study and likely important in the pathway leading to hair miniaturization in FPHL.

Table 8.1 Differences between SA and AGA

	AGA	SA
Onset	Teens	After the age of 60 years
Distribution	Patterned	May be more diffuse
Pathophysiology	DHT	Unknown
Genetics	Polygenic?	Unknown
Association or risk factor for other diseases	Cardiovascular diseases Benign prostatic hyperplasia Prostate cancer	Age-related diseases?

Is SA, like AGA, also hormonally mediated, at least to some extent? The preponderance of current evidence suggests that it is not. Biochemical studies of ARs have shown a nearly twofold decrease compared to levels in younger men with AGA; furthermore, levels of type I and II 5-alpha-reductase were only slightly higher in the frontal than the occipital scalp, suggesting that the strong hormonal signals seen in AGA are not the cause of thinning in SA [50]. In recent cross-sectional and longitudinal studies, it has been confirmed that there is progressive decline in testosterone levels with advancing age and at least 25% of men over the age of 70 years meet laboratory criteria for hypogonadism [53, 66, 74]. This pervasive waning of circulating androgens would also argue against androgen-related hair thinning. Furthermore, in early onset AGA, it has been shown that the hair miniaturization in the affected scalp can be partially reversed either by anti-androgens or by blockade of 5α-reductase with medications such as finasteride or dutasteride [25, 43, 61]. However, in older men and postmenopausal women taking finasteride, hair regrowth has not been observed, with the exception of a few case reports, again suggesting that the mechanism of thinning may not be hormonally mediated [49, 67].

It is well known that AGA tends to run in families, but the underlying genetic susceptibility and mode of inheritance has not been completely elucidated. Current evidence suggests that AGA is a polygenetic trait given the high prevalence of balding, the strong concordance between family members, and the fact that risk increases with the number of relatives already affected [5, 11, 30, 38, 56]. Various candidate genes have been analyzed as possible susceptibility markers. Linkage studies failed to find any markers for early onset AGA on chromosomes 2 or 5 which are known to code for 5α-reductase types II and I [12]. However, it has been shown that the AR gene, which is on the X chromosome plays an important role in the heritability of AGA. Specifically, frequency of single nucleotide polymorphism (SNP) in exon 1 of the AR may be measurably associated with severity of male pattern baldness [13, 14, 20]. More recently, genome-wide linkage study of 95 families with AGA has shown strong linkage to chromosome 3q26, which may provide the first step toward the identification of new susceptibility genes [21]. Similar genetic analysis of SA has, to our knowledge, not been undertaken.

With the dramatic accumulation of knowledge on the normal physiology of the folliculo-sebaceous unit, it has become clear that there are a number of highly complex and orchestrated signals that allow for growth and regulation of this unique tissue [59]. Therefore, it is plausible that there are a number of different and perhaps overlapping molecular pathways involved in control of hair growth and these may all lead to the final common pathway of hair thinning due to either follicular dropout or miniaturization [8]. Since these pathways have not been fully elucidated, we have constructed a conceptual framework of hair thinning in AGA, FPHL, and SA (Fig. 8.1) based on our current understanding of these disorders first for clarification of our nomenclature and also for the purpose of hypothesis generation. We propose that in AGA, androgens are the predominant influence on follicular miniaturization in individuals who are genetically susceptible to AGA; however, in later decades, other signals such as those involved in aging may also influence the growth characteristics of the follicle and such signals may overlap with or supersede androgen influences. The degree of "susceptibility" to either androgens or senescent signals is likely genetically determined, with individuals having a tendency to one, both, or neither. In women, early onset hair thinning is a hereditary androgen-driven condition, whereas the onset of hair loss in mid-aged women may have other or additional hormonal influences on hair growth. Thus, the possible pathways that could lead to hair thinning in women may be even greater than in men.

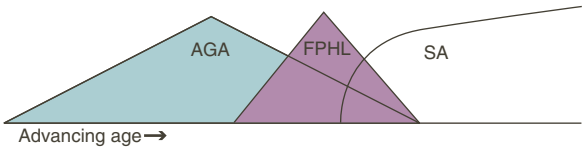

Fig. 8.1 Conceptual framework of hair thinning in androgenetic alopecia (AGA), female pattern hair loss (FPHL), and senescent alopecia (SA) based on our current understanding of these disorders

Using a molecular approach with microarray genechip technology, we have attempted to both validate prior observations and also support our hypothesis that SA is a distinct process from AGA and is not hormonally mediated. Furthermore, we identify specific senescent or aging signals and pathways that are attractive candidates for further study in hair follicles.

8.2 Comparative Gene Expression Profiling of AGA and SA by Microarray Analysis

Improved knowledge of the changes in gene expression associated with AGA and SA may lead to a greater understanding of the underlying mechanisms of hair thinning and the development of possible intervention strategies. The advantage of microarray technology is that it provides whole-genome views rather than gene-by-gene information and also offers a method for rapidly establishing potential associations between genes and functional pathways. We therefore used gene expression profiling to understand the molecular pathogenesis of AGA and SA.

All men seen in the Dermatology Clinic at the Veterans Medical Center in Cleveland who were over the age of 60 were eligible to participate in the study. The study was approved by the local Institutional Review Board. Exclusion criteria included history of scalp or hair disorders and use of known medication that may cause hair growth alteration. Three groups of men were analyzed: *Group 1 – Controls* ($N = 10$) had no visible hair thinning. *Group 2 – AGA* ($N = 10$) had male pattern hair thinning that was established to have occurred prior to age 30 and *Group 3 – SA* ($N = 10$) had diffuse hair thinning that had its onset after the age of 60. Four millimeter scalp biopsies were obtained from vertex scalp or at the leading edge of alopecia that was hair bearing, in accordance with usual clinical practices. The specimens were snap frozen in liquid nitrogen and stored at –80° prior to analysis. Gene expression profiles of pooled AGA, SA, and normal controls were generated by hybridization to Affymetrix HG-U133 Plus 2.0 chips and the data analyzed with GeneSpring (Agilent Technologies) and Ingenuity Pathways Analysis (www.ingenuity.com) software programs.

8.2.1 Differentially Regulated Genes in AGA

In AGA, 1,708 genes were differentially expressed compared to SA and the control group. Of these, 913 were upregulated (Fig. 8.2a) and 795 genes were downregulated (Fig. 8.2b) compared to SA and normal controls.

As shown in Table 8.2, hair keratins, keratin-associated proteins, and genes involved in hair follicle cycling were downregulated in AGA. Hair keratins and keratin-associated proteins are major components of the hair fiber and play crucial roles in forming a strong hair shaft through a cross-linked network with keratin intermediate filaments (KIF) [54]. In addition, seventeen genes necessary for hair growth cycling, including those required for catagen (BDNF, BMP2, BMP7, VDR, IL1, ER) [4, 16, 22, 39, 46] and telogen induction and maintenance (VDR, RAR) [24] were upregulated in AGA. Twenty four hair growth cycle genes including those required for anagen onset (Wnt-β-catenin, TGF-α, TGF-β, Stat-3, Stat-1) [45, 63], epithelial signal to dermal papilla (PPARδ, IGF-1) [3], hair shaft differentiation (Notch, Msx2, KRTs, KAPs) [32, 70], and anagen maintenance (Msx2, Activin, IGF-1) [37] were downregulated in AGA.

Since hair follicle growth cycle depends on a delicate balance between cell proliferation and apoptosis, a number of growth factors, growth factor receptors, transcription factors, apoptotic genes, and nuclear receptors such as retinoic acid receptor and PPAR delta that are required for anagen phase of the growth cycle were also upregulated in AGA. AR gene expression was also upregulated in AGA thereby supporting previous observations that these receptors play an important role in the pathophysiology of the disorder.

These gene expression changes suggest a deregulation of hair follicle cycling in AGA that may cause an increased ratio of catagen/telogen to anagen hair follicles in AGA thereby causing hair thinning.

8.2.2 Differentially Regulated Genes in SA

We identified 431 differentially expressed genes in SA, of which 164 were upregulated (Fig. 8.2c) and 267 were downregulated (Fig. 8.2d), compared to AGA and control samples. As shown in Table 8.3, SA showed large reductions (twofold or more) in the expression of genes involved in protein turnover, such as the 26S proteasome component PSMD7, ubiquitin-conjugating enzyme E2H, and the ubiquitin ligase complex RFFL, all of which are involved in the ubiquitin-proteasome pathway of protein turnover [72]. The downregulated genes in SA also play a role in actin cytoskeletal dynamics (DST,

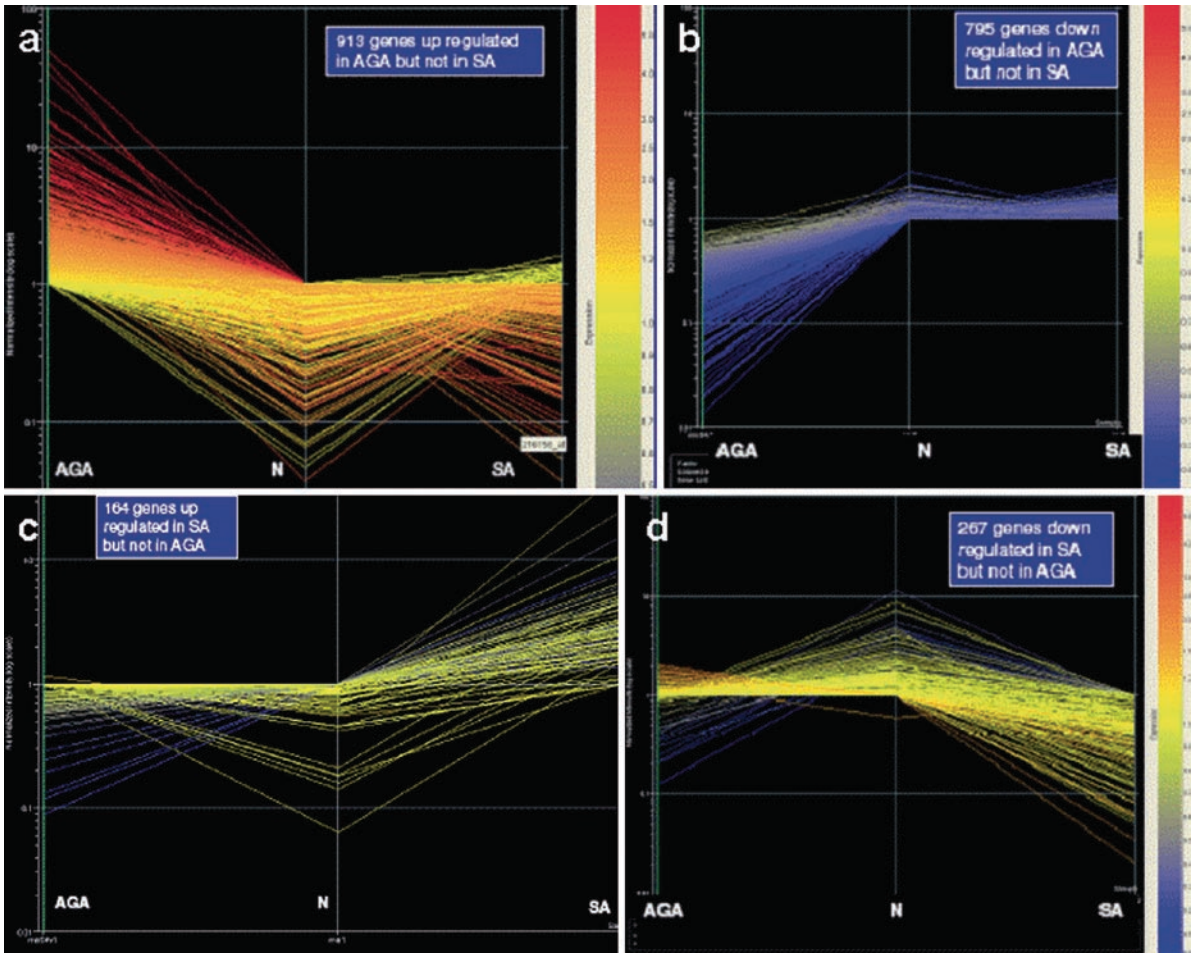

Fig. 8.2 Distribution of genes showing differential expression between AGA and SA compared to normal controls using Genespring® 7.2 software. The distribution of relative expression values for all cDNA elements is shown on the right of each panel. (**a**) 913 genes showed increased expression in AGA but not in SA compared to normal controls and (**b**) 795 genes showed decreased expression in AGA but not in SA. (**c**) 164 genes showed increased expression in SA but not in AGA and (**d**) 267 genes showed decreased expression in SA but not in AGA compared to normal controls

ACTN2, TNNI3, and PARVB), mitochondrial function, and energy metabolism (JAK2, PRKD3, AK2, TRAP1, TRIO, ATP12A, MLL4, STK22B). A number of transcription and translation factors, tumor suppressor genes, and developmentally regulated genes also show decreased expression in SA. In contrast, the upregulated genes in SA included inflammatory and stress response genes, transporters, nuclear orphan receptors, and genes involved in apoptosis.

A striking fact about the transcriptional profile of SA is that it is very similar to the transcriptional profiles of other aging mammalian systems such as aging human kidney, muscle, hippocampus, and oocytes [31,34, 51, 60,75]. This suggests that the hair loss in SA is a consequence of the aging process in the hair follicle.

8.3 Discussion

In the past two decades, dramatic advancements in the field of hair biology have elucidated many of the molecular signals and protein interactions that are responsible for the unique functionality of the follicular unit. Our microarray data shows that AGA is

Table 8.2 Gene expression changes in AGA Differentially expressed genes = 1704

Downregulated in AGA = 795	Upregulated in AGA = 913
Hair keratins and keratin-associated proteins *KRT16, KRT23, KRT32, KRT34, KRT37, KRT72, KRT83, KRT33B, KRT81, KRTAP5–9*	Nuclear and other receptors *AR, VDR, NR2F2, COUPTFII, ESRR, IR, EGFR, SDFR1, SDFR2, CHRM3, BMPR2, TLR10, TGFBR3*
Hair growth cycle *WNT, B-CATENIN, TGF-A, TGF-B, STAT-3, STAT-1, PPARΔ, NOTCH, MSX2, ACTIVIN, IGF1*	Hair growth cycle *BDNF, BMP2, BMP7, VDR, IL1, ER, VDR, RAR*
VDR-RXR signaling *CLND1, CREM, CYCLIN A, CYCLINE, CYP7B1, DNAJA, DNAJA2, DNAJA4, DNAJA5, DNAJB6, E2F, EIF5A, GOLT1B, HDAC, HIRA, HISTONE H3, HLA DQA1, HSP70, HSPA9, HSPA14, HSPA1B, KRT81, MAT2A, MAX, MRC1, RBBP4, SMARCC1, SP1, SUPT16H, SWI-SNF, SYNE2, TGM1, TK, USP6NL*	Apoptosis *AKAP, AKAP1, AKAP7, AKAP13, ATF7, CFTR, CRISP3, CX3CL1, DBT, FBXW7, GLO1, GOPC, HLA-F, IFNAR2, KRT18, KRT19, NFkB, NIBP, NOTCH4, Pka, S100A1, SHANK2, SLC4A4, SLC4A7, Sox, SOX4, SOX10, SOX15, SOX5 TACR1, TLR10, TNFSF13, TRAF4, TTN, USP7*
Chromatin remodeling *HDAC, HISTONE H3, SWI-SNF* Nuclear receptors *RAR-ALPHA, PPAR-DELTA*	Inflammatory genes *15-Lipoxygenase, ARTS-1, CCL18, CCL23, CD276, CENPB, CMIP, CNR2, CTDSPL, CYSLTR2, EOMES, FAAH, FBXO9, FLRT2, FOXN3, GPM6B, HLX, HRSP12, ICOSLG, IFNG, IL4, inosine, IPPK, ISG20, MRPS10, NT5C2, OPHN1, PELI1, RB1, RIPK1, SCGB3A1, SLC2A11, SRF, STK11, VTCN1*

associated with decreased expression of genes required for anagen onset and maintenance and increased expression of catagen and telogen inducers. This suggests that the driving force for hair thinning in AGA is deregulated hair follicle cycling. In stark contrast, hair thinning in SA is likely the result of mitochondrial dysfunction and oxidative stress. These marked differences between AGA and SA confirm that they are two distinct clinical disorders and present diverse mechanisms for a final common phenotype of follicular downsizing.

Since the hair undergoes continual and dramatically varying cycles of growth, resorption, and regeneration, it has been considered an ideal tissue for studying issues such as aging and oncogenesis. A mounting body of evidence is linking mitochondrial defects and

Table 8.3 Gene expression changes in SA Differentially expressed genes = 431

Downregulated in SA = 267	Upregulated in SA = 164
Actin cytoskeleton *D2L1C, ARHGEF10, DST, ARGBP2, DES, RAPGEF2, ACTN2, FYB, TNN13, KRTHA7, CALD1, DIAPH2, PHACTR1, SMAP1, ARFGEF2, MTSS1*	Inflammatory response *PTGER3, PTGDS, CYTL1, ANXA1, PBEF1, DAF, GBP6, LTF, RGS1, KCNN4, NR4A2, MS4A1, SOCS3, HLA-DR4, LILRB1, ANXA1, CD69, CRISP3, LYZ, PPBP, IGHA2, MUC7, DCD, MMP12*
Proteasome-ubiquitin system *RFFL, SH3MD2, BEAN, UBE2H, FBXO27, IBRDC2, FLJ31951, PSMD7, SENP6, FBXL7, TTL, DTX3*	Oxidative stress response *GADD45b, DUSP1, BTG2, FOS, CYR61, WWOX, EGR1*
ATP-binding and energy metabolism *DHX36, JAK2, ZAK, ATP12A, KIAA1387, AK2, ATP6VIG2, ATP8A2, ADRBK2, HORMAD1, PAX6, TRAP1, WNK4, MLL3, TRIO*	Transporters *HBA1, HBG1, HBG2, HBB, ATP6VIB1, TCN1,TM4SF11, SLC14A1, SLC1A1, GRIA2*
Transcription factors *ELF2, TCF7L2, MAF, DACH1, MED6, NFIC, MYOCD, MLL3, CNOT7, ETV6, PAX8*	Apoptosis *FADD, NF-kB, JunB, FosB, NR4A2*
Translation factors *EIF5A, ANKHD1, EIF3S9, EIF4G3*	Nuclear orphan receptors *NR4A2, NR4A1, NR1D1(THRA), ESRRG*

age-related physiologic changes [10, 19, 33]. Previous studies [71] with mitochondrial DNA (mtDNA) mutator mice have shown that increased somatic mtDNA mutations cause a variety of aging phenotypes including hair loss and graying of hair. Mitochondrial respiratory chain deficient cells are apoptosis prone and increased cell loss is therefore likely an important consequence of age-associated mitochondrial dysfunction [76].

The free radical theory of aging, first proposed in the 1950s suggests that oxidative damage accumulates in cells and tissues over time and contributes to the decline in physiologic function with age [69]. Since the mitochondrial electron transport chain is the major site of production of reactive oxygen species, it has been proposed that mitochondria are the prime target of oxidative damage. Mitochondria are an attractive "suspect" for contributing to the aging process, because the mitochondrial genome has a number of unique features including the presence of "naked" DNA unprotected by histones, close proximity of the mtDNA to the respiratory chain, and decreased repair capacity compared to nuclear DNA [10]. Tissues with high oxidative requirements such as muscle, heart, and brain have been shown to be the site of increased mitochondrial defects [10]. However, mitochondrial defects have also been shown in other aged tissues, specifically in the skin [10]. Furthermore, patients with inherited mitochondrial disorders have been reported to have skin and hair abnormalities [7, 29, 55].

Another set of genes downregulated in SA are the actin cytoskeletal genes. The actin cytoskeleton plays a fundamental role in many cellular processes and maintains the integrity of all the membranes in the cell. The decrease in ATP production and energy pathways in SA suggests a decrease in mitochondrial function. In yeast cells, the actin cytoskeleton cooperates with the mitochondria to regulate the production of reactive oxygen species [15]. Decrease in actin cytoskeleton increases the production of ROS and induces aging in yeast. The increased expression of oxidative stress responsive genes further supports the possibility of an oxidative damage of the cellular components of the hair follicle in SA.

An intriguing observation we made with SA is the downregulation of genes involved in the protein ubiquitination pathway. Aging is characterized by accumulation of potentially harmful altered proteins that could lead to gradual deterioration of cellular functions and eventually result in increased probability of cell death. Protein activity and turnover are closely tied to posttranslational modifications like ubiquitination, which target proteins for proteosomal degradation and other myriad cellular functions [68]. Thus, loss in proteasome function may impair the ability of hair follicle cells to mount an appropriate response to oxidative stress, thereby enhancing susceptibility to hair thinning.

A potential consequence of altered protein accumulation is the induction of an inflammatory response. During aging, changes in proteins occur that alter their function and render them immunogenic. For example, oxidation accelerated aging of red cells is reported to generate senescent cell antigen and IgG binding, and triggers removal of red cells by macrophages [26]. Thus in SA, we observed that genes involved in prostaglandin synthesis, complement activation, and B and T cell activation showed increased expression. Similarly, an increased inflammatory response has been reported in other aging systems.

In conclusion, our results are supportive of the hypothesis that accumulation of mitochondrial defects in the stem cells of the hair and subsequently in the transient amplifying cells of the hair matrix may lead to apoptosis and age-related hair thinning. Thus, SA may be an accessible marker of other systemic aging processes or disease states. In the future, further elucidation of mechanisms leading to senescent thinning may provide options for prevention or treatment of hair loss.

Take Home Pearls

- AGA and SA have very different gene expression profiles suggesting that they are two distinct disorders.
- AGA – associated with decreased expression of genes required for anagen onset and maintenance and increased expression of catagen and telogen inducers.
- SA – microarray profiling identifies mitochondrial dysfunction and oxidative stress.

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The Aging Hair Pigmentary Unit

9

Desmond J. Tobin

Core Message

- As a highly visual and social species we communicate significantly via our physical appearance. Thus, it is unsurprising that the phenotypic aspects (including color) of our skin and hair feature prominently in such communication. Perhaps, one of the more potent reminders of aging is the change in pigmentation from birth to puberty and through to young adulthood, middle age, and beyond. Indeed, the hair bulb melanocyte may be viewed as an exquisitely sensitive aging sensor. In this context, we can appreciate that the loss of pigmentation from the hair tends to be earlier and much more striking than the age-associated pigmentation changes that we see in the epidermis.
- This phenotypic difference between the hair follicle and the epidermis-melanocyte subpopulations is of considerable interest, not least as both subpopulations originate from the same embyrologic neural crest and that the melanoctye stem cells in the adult hair follicle can occupy vacant niches in the epidermis. A major source of the differential aging of melanocytes in the hair bulb vs. the epidermis is likely due to the former's stringent coupling to the hair growth cycle when compared with the latter's continuous and UV-sensitive melanogenesis. Also likely to be involved is the maintenance of permissive microenvironments in these different skin compartments including their differing redox environments and variable connectivity with neuroendocrine axis.
- Over the last few years, we and others have striven to develop advanced cell culture methodologies for isolated hair follicle melanocytes and for intact anagen hair follicle organ culture, which may provide research tools to elucidate the regulatory mechanisms of hair follicle pigmentation. Others have assessed the robustness of the hair follicle-melanocyte stem compartment with age and other functional stressors. In the long term, it may be feasible to develop strategies to modulate some of these aging-associated changes in the hair follicle that impinge particularly of the melanocyte populations.

9.1 Introduction

Although the skin contains hemoglobin and carotenoid pigments, only the melanins contribute significantly to our overall skin and hair color. Melanins are a class of mixed indole-rich compounds formed via a phylogenetically ancient and complex biochemical pathway called *melanogenesis*. This biochemistry occurs in unique lysosome-related organelles called *melanosomes* that are formed within the cytoplasm of cells called *melanocytes*. While even the casual observer

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can appreciate how hair growth and pigmentation have facilitated evolutionary success in other mammals, it has been much more difficult to determine how these played a fundamental role in human survival over time. Still, as much of the human species' success is due to its social prowess, it is reasonable that skin and hair color would contribute significantly in human social communications as these traits provide much information regarding an individual's health, age, ethnicity, gender, and attractiveness. More enigmatic still is the luxuriant and pigmented nature of human scalp hair, which uniquely among the primates is likely to have had important evolutionary selective pressure [52].

At least two characteristics of hair follicles may provide some clues. Hair follicles contain highly proliferative tissue, with a cell division rate second only to the bone marrow and gut epithelium [45]. The proliferative epithelial fraction of the growing or anagen hair follicle bulb matrix is almost 100%. Moreover, this massive keratinocyte proliferation can continue for extraordinarily long periods of time – a particularly famous example is a 42-year-old Chinese woman with scalp hair of 4.3 m, representing a continuous anagen of 26 or more years [38]. More usually, scalp anagen persists for 3–5 years, and these follicles extrude the hair fiber at a rate of approximately 1 cm per month. In this way, the hair, which undergoes no further biogenic change, can trap and bind-up toxic materials (e.g., heavy metals) from the scalp tissue and ultimately from the entire body via the circulation and highly vascularized hair follicles [52]. Contributing to this is the very high rates of melanogenic activity in growing pigmented scalp hair, which facilitates the selective and avid binding of toxins and metals to melanin pigment [52, 65]. In this way, the complex indoles of melanin can act as an important sink for sequestering potentially noxious materials within the long, deeply melanized, scalp hair fibers, thereby limiting their access (and so potential damage) to the living tissue of the highly vascularized scalp.

The vast majority of the global human population has dark brown/black scalp hair during their early/mid-lives, perhaps reflecting our out-of-Africa origins and the climatic imperatives associated with this [65]. However, what about the remaining 5–10% of humans concentrated today in northern Europe who have emerged with the diverse palette of colors, e.g., white blonde, yellow blonde, auburn, red, and all shades in between? The relatively recent discovery of mutations in the melanocortin-1 receptor (MC1-R) gene, which encodes for a G-protein-coupled receptor, has contributed hugely to our understanding of mammalian pigmentation [6, 35]. MC1-R is activated in the main by pro-melanogenic peptide ligands derived from pro-opiomelanocortin including α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropin hormone (ACTH) [6]. Some subpopulations of northwestern Europeans, especially those with red hair, are homozygotes or compound heterozygotes for a limited number of MC1-R mutations [34].

The first significant assessment of pigmentation in human hair follicles, which has set the scene for even very recent work, is the exceptional report "The nature of hair pigment" by Fitzpatrick et al. [13], published in 1958. In this study, I will provide only a brief review of the regulation of hair follicle pigmentation before focusing, instead on our current knowledge of the aging pigmentary unit and canities or hair graying. Interested readers are directed to the recent more comprehensive reviews on the topic [41, 42, 53, 54].

9.2 Origin of Hair Follicle Melanocytes

To appreciate the fate of melanocytes during human life and the implication this has for melanocyte stem cells, proliferation, differentiation, and death, it is important to have some appreciation of the origin of these fascinating cells. Melanocytes of both the epidermal- and follicular-melanin units are derived from immature melanocytes or "melanoblasts" that migrate along stereotypic pathways from the neural crest into the skin during embryogenesis. The regulatory controls of melanocyte lineage commitment remain a subject of intense interest in research [49], and so far it appears that microphthalmia-associated transcription factor (MITF), SOX10, Pax3, KIT, fibroblast growth factor-2, and endothelin 3 are involved to variable extents [49]. Once committed, endothelin 3 appears to be required to differentiate early melanoblast on their journey to the epidermis [66], and by 7 weeks of human gestation initiation of melanin production (melanogenesis) can be seen in these cells several weeks before actual hair follicle development commences [18]. There is an emerging view, however, that immature melanocytes even in adult skin may retain some plasticity regarding their potential differentiation trajectories [49].

At the time of hair follicle morphogenesis/development, some progeny of proliferating melanoblasts/melanocytes in the epidermis (often called "transit or transient-amplifying" melanocytes) leave the epidermis and move to the developing hair follicles. Depending on the intra-follicular compartment in which they reside, melanocytes may become/remain dopa oxidase-positive (i.e., express active tyrosinase) or remain dopa oxidase-negative cells (i.e., either fail to express tyrosinase or express an inactive tyrosinase) [31]. It is perhaps worth reflecting at this point, as such compartmentalization of follicular melanocyte subpopulations during skin development is likely to be important for considerations of melanocyte "renewal" during the hair growth cycle, role of the stem melanocyte reservoir, and during age-related pigmentation changes and age-related depletion of functioning melanocytes during graying.

9.3 Cutaneous Melanocyte Subpopulations and Implications for Hair Aging

Even indifferent observations of our fellow humans highlight the apparent independence of the epidermal- and follicular-melanin units, e.g., the co-expression of white hair and black skin in aging people of African descent and conversely the raven hair of some white Europeans. This is also reflected at the cellular level where differentiated hair bulb melanocytes tend to be larger, more dendritic, have more extensive Golgi and rough endoplasmic reticulum, and produce larger melanosomes than their epidermal cousins [54, 55, 57]. Moreover, while melanin granules are almost completely degraded in the differentiating layers of the epidermis, hair follicle melanin granules transferred into precortical keratinocytes remain minimally digested (although red/yellow pheomelanin appears to be less-resistant than black/brown eumelanin). In that way, the distal ends of a typical hair shaft remain similarly pigmented at proximal and distal ends.

Perhaps, reflective of their original migration pathways during skin and hair follicle development, the more superficially distributed melanocytes are broadly melanogenically active (i.e., with 3,4-dihydroxy phenylalanine (dopa)-oxidase activity of tyrosinase) and are detected in the basal layers of the epidermis, infundibulum (i.e., uppermost hair follicle), and sebaceous gland. It is not yet clear what role the latter melanocyte subpopulation has, although this is likely to be at least in part antimicrobial in this hormone-sensitive holocrine gland [24, 56]. Proximal to this subpopulation are scattered dopa oxidase-negative, and hence amelanotic melanocytes of the mid-to-lower outer root sheath. These follicular pigment cells may represent a pool of "transient" melanocytes that migrate from precursor melanocyte stores in the hair follicle bulge to other areas of the outer root sheath [19, 28].

The main site for the most differentiated follicular melanocyte subpopulation is the hair bulb matrix located above and around the upper follicular papilla. Although these melanocytes donate melanin directly to keratinocytes of the hair cortex and less to the medulla, and rarely the hair cuticle, this transient region of the hair follicle contains an enigmatic additional but minor subpopulation of poorly differentiated melanocytes [32, 46] distributed to the most proximal and peripheral regions of the growing hair bulb. It is possible that these represent a migratory population that maintains the bulbar complement of functioning melanocytes or they may indeed have a nonpigmentary role (cf. [51]). Thus, one of the surprises to newcomers to the field of hair follicle pigmentation is the multiple subpopulations of melanocytes in human scalp hair follicles. We and others have attempted to characterize these multiple subpopulations of melanocytes using *in vitro* strategies. While immature follicular melanocytes can be detected in primary cultures of follicular melanocytes, it is not yet clear whether some of these cells may indeed have *bona fide* melanoblast or stem cell potential [55, 58].

9.4 The Hair Growth Cycle and Its Implications for Aging of the Hair Pigmentary Unit

Despite their common origin, epidermal- and follicular-melanocyte behavior is regulated differently [32, 40, 41, 44, 58]. In this context, follicular-melanocyte activity is episodic (driven by hair cycle change), whereas epidermal-melanocyte activity is generally continuous, although this constitutive activity can be stimulated further: i.e., after exposure to UV radiation. As active hair follicle pigmentation only occurs during active hair growth (i.e., anagen), the duration of this

phase will have direct implications for follicular-melanocyte homeostasis. Thus, for scalp hair follicles bulbar melanocytes will be engaged in melanogenesis for up to 10 years, whereas active melanogenesis of less than 1 month is likely for eyebrow hair follicles. However, the so-called (blocked-in-anagen) cases, as dramatically represented by the highly pigmented and very long (4.6 M) scalp hair of Mrs Y.D. in China, force us to conclude that there may be enormous melanocyte capacity in the hair follicle pigmentary unit during a single (albeit 26 year + long) anagen [38]. This view is based however on the unproven supposition that there is little or no replacement of bulbar melanocytes during a single anagen phase. Our recent identification of a second, immature melanocyte subpopulation in the proximal bulb leaves open the possibility for intra-anagen turnover of terminally differentiated bulbar melanocytes [12].

No melanin is actively produced (tyrosinase and tyrosinase-related protein 1 mRNA and protein are undetectable) during the relatively resting stage of the hair cycle called telogen. This runt stage of the hair cycle (less than 30% of the growing hair follicle tissue mass) contains however all cell precursors needed to reconstitute a fully pigmented anagen VI hair follicle (Fig. 9.1). While it is not clear which factor(s) drive the transition of the telogen hair follicle into early anagen, these events also stimulate or subsequently trigger the re-activation of the follicular pigmentary unit. In murine hair follicles, some dopachrome tautomerase-positive melanocytic cells (melanoblasts or true melanocyte stem cells) can be detected in the telogen of follicles and some of these are also the melanocyte-survival marker KIT [29]. One to two days after anagen induction, these cells begin to express tyrosinase mRNA, and subsequently tyrosinase but not tyrosinase activity, and some of these also being to express TRP1. Melanocytes residing in the upper outer root sheath (site of the presumptive germ cell reservoir) however remain TRP1 negative, whereas a second TRP1/DCT/KIT-positive melanocyte subpopulation now begins to proliferate [13].

A critical determinant of melanocyte behavior is its local microenvironment. Thus, it is striking that only follicular melanocytes located close to the anagen follicular papilla, which appears to be a permissive location. This does not appear to involve a generic mesenchymal signal, as melanocytes in the outer root sheath closely associated with the mesenchymal

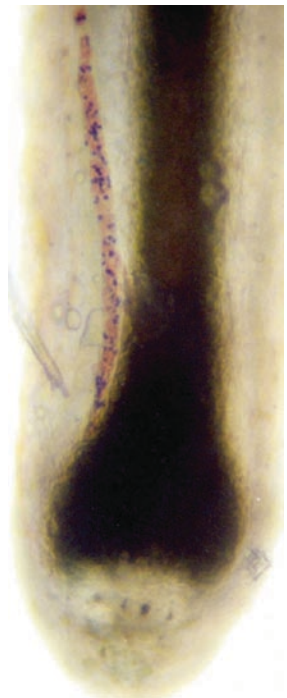

Fig. 9.1 Fully pigmented human anagen scalp hair follicle showing intense melanization of the hair bulb and hair shaft and prominent blood vessel (*left*)

dermal or connective tissue sheath remain for the most part amelanotic [19, 51]. The follicular papilla cells exhibit a biochemical milieu that favors the production of high amounts of L-tyrosine from L-phenylalanine [37, 65]. A particular redox environment appears to exist in the follicular papilla that is pertinent to the support of melanogenesis. This is perhaps to be expected given that reactive oxygen species (ROS) are themselves produced as a function of melanogenesis (cf. [37]). This latter point has implications for canities, as oxidative stress may play an important role in hair graying.

It appears that the telogen–anagen transition is the only period of the hair cycle in which follicular melanocyte proliferation occurs. Proliferating melanocytes can be first detected during anagen II, with a subsequent burst of melanocyte mitosis later in anagen III [46]. Hair bulb melanocytes reach a peak by anagen VI or full anagen stage that is characterized by maximal hair fiber production. Daughter melanocytes thereafter differentiate with become larger and more dendritic in preparation for the active transfer of mature melanosomes to precortical keratinocytes in full anagen.

The transition between high anagen and the start of catagen-associated regression of the hair follicle is also the subject of intense study. During this event, the

activity and expression levels of tyrosinase decreases rapidly to become undetectable/very low in late catagen [40, 60]. It is during this phase of the cycle that several follicular melanocytes depart significantly from the regulatory norms operating in the epidermal-melanin unit. This catagen-driven physiologic decrease in follicular melanogenesis may reflect either an exhaustion of an active signaling system that stimulates melanogenesis, and/or may be caused by the production of inhibitors of melanocyte activity. It is of note that melanocyte change can even predate final cessation of keratinocyte proliferation, with early melanocyte effects including retraction of cell dendrites [60], as a result there is a brief "canities-like" event of unknown function at the end of each hair cycle: i.e., a limited amount of keratinocyte proliferation continues for a short while such that the last section of hair shaft produced is usually unpigmented. Equally curious is the production of pigment at the end of anagen, which fails to be incorporated into the hair shaft, but is instead distributed to the follicular papilla, epithelial strand, or connective tissue sheath of these catagen hair follicles. Similar pigment incontinence is also seen during canities, again highlighting some similarities between hair bulb melanocyte fate during catagen and canities (see below).

A further change in the redox status of the regressing hair follicle, and particularly its associated follicular papilla, may contribute to the altered follicular-melanocyte status at this time. Specifically, DCT activity also drops significantly to its lowest levels during catagen, and this tautomerase has been reported to improve melanocyte survival to oxidative stress [26]. Moreover, pterin (e.g., $6BH_4$) synthesis and PAH activities are at their lowest during catagen, and a reducing environment nonconducive to melanogenesis is provided by rising the levels of thioredoxin reductase. This reduces the supply of L-tyrosine to the hair follicle pigmentary unit, further generating conditions unfavorable for active melanogenesis [37].

Recently, we provided formal proof to explain disappearance of mature hair bulb melanocytes during catagen. The prevailing view was that the hair bulb melanocyte system was a self-perpetuating system, whereby melanocytes involved in the pigmentation of one hair generation remain involved in the pigmentation of the next and so resisted catagen-driven apoptosis in the hair bulb [9]. In contrast, our current view is that the need for melanocyte replacement from the melanocyte reservoir in the upper hair follicle [28, 60] is driven by the loss of at least a proportion of the mostly highly melanotic (and possibly terminally differentiated) hair bulb melanocytes by apoptosis [58]. It is possible, however, that some melanogenically active melanocytes derive from a subpopulation of catagen-surviving melanocytes [9], although these may themselves be lost via apoptosis in the next hair cycle change-over. The above view depends on the existence of a flexible hair follicle melanocyte "stem" reservoir, and these are located in the permanent part of the cycling hair follicle [28].

9.5 Aging of Melanocytes and Its Implications for the Aging Hair Follicle Pigmentary Unit

While the jury may be out on whether we can gain or loose significant numbers of hair follicles during our lifetimes, there is no doubt that they can dramatically change their form during life. A single hair follicle can produce a fine (un)pigmented lanugo hair during fetal life, change to a shorter unpigmented vellus hair during childhood, which thereafter can increase to less fine pigmented intermediate hair in the prepubescent child. At puberty, these same follicles are able to produce longer, thicker, and most pigmented terminal hair shafts characteristic of the adult. Miniaturization of hair fibers (e.g., in androgenetic alopecia) may represent a partial reversal of this sequence later in life [1].

As alluded to above, the hair follicle pigmentary unit also is highly susceptible to age-related change, particularly visible in those of European ancestry. Broadly speaking, hair color is at its lightest in early childhood and become progressively darker even before puberty and darkening further during adolescence and young adulthood [23] until the onset of hair graying or canities. Hair color darkening with age is largely due to the influence of hormones, including the sex steroid androgens and estrogens. We and others have also shown that melanocytes are acquisitively sensitive to (neuro)endocrine factors [41, 56]. There also are significant body-site variations in regulation of hair color, which may reflect significant local variations in hormonal stimulation of hair follicles, and these too can become more visibly expressed at certain ages. A striking example of this effect is the phenomenon of heterochromia (i.e., two-tone hair color) that

also becomes more apparent with age and is often strikingly apparent for scalp and beard (e.g., brown scalp and red beard), but may also affect the scalp alone [63].

The age of 30 years is a useful watershed period to assess the effects of chronologic skin aging in humans – perhaps reflective of the period beyond which change is not likely to have exerted significant evolutionary (i.e., reproductive) effect. Based on this consideration alone, it is difficult to predict any functional advantage for gray or white hair, beyond some social and communication signaling value.

The stable (i.e., constitutively active) epidermal-melanin unit succumbs to a 10–20% reduction in pigment-producing epidermal melanocytes (whether in sun-exposed or unexposed skin) for every decade after 30 years of age [41, 63]. Given the extremely low turnover of melanocytes in the epidermis (even after UV exposure), this would appear to indicate that epidermal melanocytes are relatively long-living cells, which must be well protected (e.g., via BCL2 expression) from exogenous stressors including UV radiation-induced ROS, and endogenous oxidative stress hose generated during melanogenesis itself.

At this point, it may be useful to explore our current understanding of how melanocytes age, although the caveat here is that most data derived from *in vitro* studies refer to studies, where a form of enforced proliferative state and replicative senescence is induced. Thus, it is not at all clear how these data may be accurately extrapolated to melanocytes *in situ*.

9.6 Molecular Mechanisms of Melanocyte Aging

The systematic study of melanocyte aging has only begun relatively recently (cf. [57]), and has been further stimulated by the significant recent interest in melanocyte stem cells – particularly how these cells may play a role in both hair graying and melanoma [14, 20]. Melanocytes are lost with age loss, not only from the skin (epidermis and hair follicle), but also from nevi and the eye. Most attention has however been directed to the loss of the easily detected dopa-positive (i.e., tyrosinase-positive) melanocytes (Fig. 9.2), although it has been inferred that total graying must also involve some loss of melanocytes from the stem cell reservoirs.

The rate of melanocyte loss may vary between different body sites and within a compartment of the same body site. This heterogeneity may reflect differences in their intrinsic "melanogenetic clocks" or rather a deficit or loss of permissive microenvironments. Thus, loss of pigment is rather gradual in the epidermis, while age-related loss in the color of hair can be more dramatic. Heredity appears to be a dominant factor and

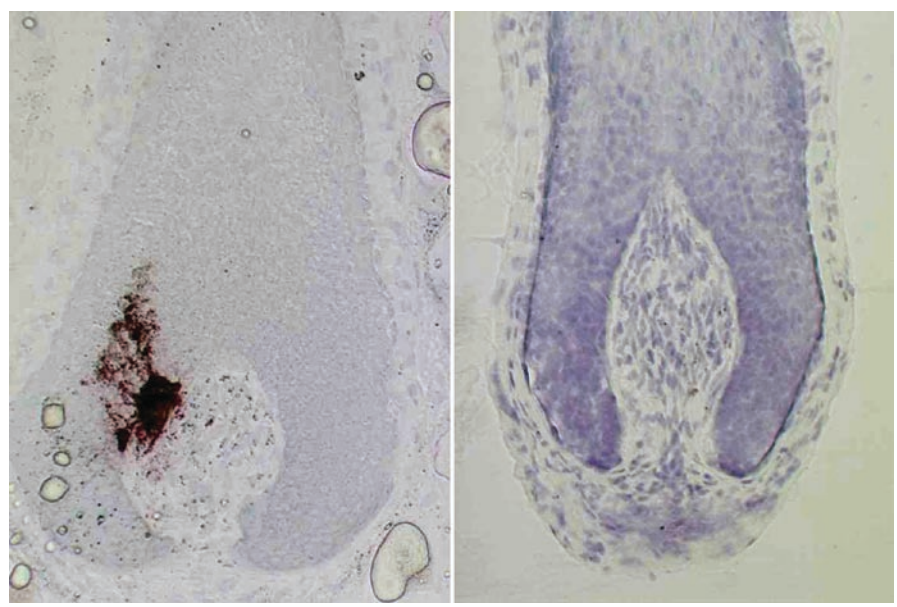

Fig. 9.2 Loss of melanocytes from the hair bulb of aging human anagen scalp hair follicles. *Left image* shows a defective and hypertrophic melanocyte stained with an antibody to gp100. Note the large size of this melanocyte filled with melanin and with abnormal denricity. *Right image* shows absence of any detectable melanocytes in the anagen hair bulb

much of canities appears to be inherited in an autosomal-dominant manner. The latter would explain the fact that entire extended families can experience marked early graying or unusually later graying. There may be variation in the size of melanocyte reservoirs in different tissues and between different individuals, which may reflect different rates of "seeding" from the neural crest during melanocyte precursor migrations during embryogenesis. There are several congenital conditions characterized by aberrant migration of melanocyte precursors along stereotypic routes, e.g., piebaldism [43].

Beyond these observations and some more recent findings in mice, we know relatively little [29]. While the precise mechanism(s) of melanocyte loss in both the epidermis and hair follicle is unclear, it is likely that the hair cycle will exert significant effects to explain observed differences in the aging of these two skin melanocytes subpopulations. In this regard, the recent formal identification of melanocyte stem cells in the upper mouse hair follicle [28, 29] will shed some light on the fate of their progeny in the epidermal and follicular melanin units during the life in humans. One view to emerge is that melanocyte aging related to hair graying may involve defective maintenance of melanocyte stem cells. If the anti-apoptotic protein Bcl2 is deficient, this loss of melanocyte stem cell is accelerated via apoptosis when they enter their dormant telogen state [29]. Nishimura and co-workers also suggested that physiologic aging of melanocyte stem cells may lead to the presence of ectopic pigmentation (or differentiation) within the stem compartment. Deficiency in the melanocyte master transcriptional regulator Mitf-M may also be involved in this process. More recently, this view was developed further by the demonstration in mice that accumulation of irreparable DNA damage with age (e.g., by ionizing radiation) may block melanocyte stem cell renewal [20]. If fact, this study rather showed that such a stress drives the stem cell to differentiate and thereafter to be lost from the niche rather than inducing senescence or death of the stem cells. A potential role for "ataxia–telangiectasia mutated" (ATM) as a checkpoint molecule for stemness was suggested. The net effect is a lack of melanocyte stem cells to replace differentiated melanocytes lost from the catagen hair bulb of the new anagen phase.

Another way to examine how melanocytes age is via manipulation of these cells in vitro. Epidermal and hair follicle melanocyte culture methodologies [12, 58] provide a useful and accessible tool to examine differences in the aging of these closely related skin-melanocyte subpopulations in humans. Examination of the primary cultures revealed greater proliferation in the more immature melanocytes (i.e., less differentiated cells). The loss of melanocyte replicative potential *in vitro* is associated not only with increasing age of the donor, but also is linked with the melanocyte's ability to process melanin within the cell and with its *in vitro* age. The long-term continuous exposure of melanocytes to cAMP inducers (e.g., cholera toxin) induces both pigment production and a so-called "pre-senescent" stage. This signaling also does not directly stimulate the MC1-R as would be expected to occur more naturally.

Moreover, cAMP induction of this type also caused the cells to become large, epithelioid, and stellate. A similar effect was observed when epidermal melanocytes were incubated with high levels of L-tyrosine (a melanin precursor), i.e., mitosis continued only in non-responsive amelanotic cells but was blocked in melanocytes that showed "pre-senescent" pigmented morphology [25]. Further studies revealed that the MAP kinase pathway was resistant to activation in these "pre-senescent" melanocytes resulting in their failure to proliferate [4]. Other features of these post-mitotic melanocytes included increased expression of cyclin-dependent kinase (CDK) inhibitors (e.g., p21 and p16), and their binding to CDK4, which inhibit cell cycling [3]. Other features of replicative senescence in normal melanocytes include increased binding of CDK-I p16(INK4a) to CDK4, downregulation of cyclin E (and so loss of cyclin E/CDK2 activity), retinoblastoma protein RB under-phosphorylation (and so increased levels of E2F4-RB repressive complexes), and progressive telomere shortening [3]. However, unlike the situation in fibroblasts, the CDK-Is p21(Waf-1) and p27(Kip-1) are downregulated. That these changes are not seen when melanocytes are induced to over-express the catalytic subunit of the enzyme telomerase (hTERT) suggests that they are indeed important for replicative senescence. How important this melanocyte-associated deviation from the molecular mechanisms found in fibroblasts is not yet clear, although it is likely to reflect the very different microenvironments of these two cutaneous cell types with their different requirements to regulate the cell cycle in response to telomere attrition and thus prevent transformation.

These early observations above have led to a flurry of theories that claim to explain age-related change in melanocytes. The dominant theory "borrowed" from mainstream aging research is the "free radical" theory of aging [15, 16], where the accumulation of oxidative damage determines the rate of aging. However, like several aging theories, it is not at all clear whether they adequately address the primary cause(s) of aging. For example, a failing melanocyte could be expected to exhibit a raft of "free-radical"-associated anomalies, although these *per se* may not have *set* the cell on the road to degeneration.

Even if the production or the presence of ROS in aging melanocytes tells us little about their origin, their targets for injury provides insight into mechanism. Clearly, ROS can damage biomolecules and their effects on DNA (nuclear and mitochondrial) can be particularly devastating in that these lesions can drive an accumulation of mutations. However, cells are usually equipped with significant resources to combat the effects of oxidative stress, for example, by a robust stimulation of antioxidant mechanisms. Increasing evidence suggests that aging impairs the cell's ability to mount a robust antioxidant response, such that increasing impairment with age can lead to uncontrolled damage to the melanocyte itself. The situation for melanocytes may in this regard be much worse than that for other skin cells, as much of the business of melanocytes is to produce melanin via a biochemical pathway replete with melanogenesis-related oxidative stress, e.g., quinone and semi-quinone production (see below) [17].

A recent study by the Peter's laboratory has reported that the follicular–melanin unit of graying hair is indeed associated with increased melanocyte death by apoptosis and oxidative stress [2]. Interestingly, the "common" deletion in mitochondrial DNA (a marker of oxidative stress) was found to occur more prominently in graying hair follicles than in matched normally pigmented hair follicles. The interpretation here therefore is that graying hair follicles in this study were also less well equipped to handle exogenous oxidative stress, and that this was likely due to their impaired antioxidant systems. We need to be a little careful however how we interpret such findings as it has also been shown that the growth rate of hair fibers in gray/white hair follicles can be significantly greater than for matched pigmented hair follicles both *in vitro* [21] and *in situ* [27, 62]. Therefore, active keratinocyte proliferation was facilitated in this tissue environment, and these cells may either express intact antioxidant systems or are more resilient in the presence of oxidative stress than melanocytes. A recent study by Schallreuter's laboratory used FT-Raman spectroscopy *in vivo* to demonstrate that human gray/white scalp hairs accumulate hydrogen peroxide in millimolar concentrations [65]. The scalp tissue revealed little catalase and methionine sulfoxide reductase A and B protein expression, and there was a functional loss of methionine sulfoxide (Met-S = O) repair in the affected hair follicles. This environment damages tyrosinase function. However, it is not yet clear whether these changes are causative in the loss of hair pigment in graying hair follicles.

9.7 Is the Very High Melanin Production Rate of Hair Melanocytes a Problem?

By comparison with other cutaneous melanocytes, hair bulb melanocytes during anagen exhibit both a phenomenally high capacity for melanin production and a very high intracellular melanin load. Indeed, a relatively small number of melanocytes, perhaps as few as 100 cells per scalp anagen hair follicle can, in a single hair growth cycle, produce enough melanin to intensely pigment hair fibers of up to 1.5 m in length. Recently, assessment of the anagen-block hair follicles seen in exceptional cases of long scalp hair (i.e., up to 4.6 m during an anagen of over 26 years [38]) suggests that these cells can maintain this melanin synthetic rate even longer, provided they reside in an anagen-associated permissive microenvironment.

It is not clear why the cytoplasm of bulbar melanocytes should contain a much higher fraction of mature melanosomes than that occurs in the epidermal melanocytes. There is no evidence that melanin transfer to the surrounding keratinocytes is more efficient in the epidermis. Indeed, the 1–1.5 cm/month production of pigmented hair fiber is as impressive, if not more so, that the monthly turnover of the much thinner human epidermis. The prolonged melanogenesis characteristic of hair bulb melanocytes during anagen is likely to generate large amounts of ROS via the oxidation of tyrosine and dopa to melanin. If these ROS are not efficiently removed, the resultant oxidative stress would damage the melanocyte itself, and pose significant potential risks for mutation. This would be prevented if melanogenic bulbar melanocytes assumed

a postmitotic, terminally differentiated "(pre)senescence" status.

9.8 Histopathology of Hair Graying (Canities)

At its simplest, the gray hair follicles have markedly reduced numbers of differentiated and functioning hair bulb melanocytes, whereas the white hair bulb may have none at all. The focus here is on the hair bulb, although the retention of some melanocytes elsewhere in the hair follicle for some time after graying of the hair fiber remains open in my view, despite some more fixed positions emerging in the literature [10]. The precise mechanisms responsible for the loss of melanogenically active melanocytes from anagen adult hair follicles with increasing age remain rather speculative. In true gray hair follicles, melanin granules can be readily detected within the precortex and the hair fiber, and often in an asymmetric pattern. Gray hair bulbs exhibit a much reduced, yet detectable, dopa-oxidase reaction indicating that melanocytes remain with at least some tyrosinase activity. In this way, gray hair follicles are a potentially interesting test system to examine the process of melanosome transfer from the melanocyte partner's perspective, as residual melanocytes in these affected hair bulbs often show blunted dendrites with clearly defective melanosome transfer in the graying hair follicle. Moreover, surrounding precortical keratinocytes fail to receive melanin granules from these damaged melanocytes despite their still moderate levels of mature melanosomes [51, 53, 57]. In this sense, the normal relationship of melanocyte to keratinocyte in the follicular melanin unit breaks down. In contrast, white hair bulbs are broadly negative for the dopa-oxidase reaction [53, 57].

The somewhat "messy" loss of integrity of follicular melanin unit is suggested by significant melanin debris/incontinence within and around graying hair bulbs. In this sense, graying hair follicles resemble some of the changes associated with breakdown of the follicular melanin unit in catagen hair follicles [9]. Degenerating bulb melanocytes in graying follicles exist in this dystrophic hypertrophic state for some time. When the intracellular environment of these degenerating melanocytes is examined, it is clear that the compartmentalization of melanogenesis has become dysregulated. This can be seen in the packaging of melanosomes within auto-phagolysosomes, suggesting that the defective cells are attempting to remove defective (leaky?) melanosomes. If indeed leaky, these melanosomes may release reactive oxygen metabolites derived from the melanin bio-synthetic pathway into the cell cytoplasm and trigger autophagolysosomal degradation. If the cell is unable to efficiently remove defective cell constituents such as melanocytes, the cell itself may become compromised and die [59, 60]. Evidence of melanocyte death in canities can be seen in levels of ectopic melanin redistributed to the follicular papilla and/or connective tissue sheath of hair follicles that lack any evidence of intact melanocytes or active melanogenesis. The most reasonable interpretation of these findings is a very recent loss of melanin/melanocytes from a previously melanogenic hair follicle.

Defecting compartmentalization of the biochemically highly reactive process of melanogenesis together with reduced or inefficient antioxidant system may lead to increased availability of ROS in the melanocytes. Further support for the involvement of ROS in the histopathology of canities is suggested by the observation that melanocytes in graying and white hair bulbs may be vacuolated, a common cellular response to increased oxidative stress. Indeed, there is a correlate here with another pigmentary anomaly, i.e., loss of functional epidermal melanocytes in vitiligo. In the latter, millimolar levels of the oxidant H_2O_2 can be found, which damages the epidermal melanocytes [61]. However, unlike vitiligo, melanocyte loss in canities does not appear to trigger a nonphagocytic immune system response. Rather, in canities melanocyte degeneration leads to the removal of degenerative melanogenic melanocytes from the hair bulb of graying and white hair follicles. This "clean-up" process may be associated, however, with an increase in dendritic cells including Langerhans cells [50], attracted to this immune-privileged part of the hair follicle [8] by stimulation from degenerating canities-affected melanocytes.

9.9 Melanocyte Loss from the Aging Hair Follicles and Its Impact on the Hair Follicle

Melanin production by hair follicle melanocytes is much more limited than keratin production by the hair follicle keratinocytes. The latter has been shown to

continue successfully for over 110 years without fail for these long-living individuals, a feat which may infer a greater integrity of both keratinocyte replacement and keratin production. While melanocytes are lost from hair follicle throughout the body, the effect is more striking on the scalp. Moreover, there may be some region-specific differences in the handling of stress by melanocytes and melanocytes stem cells in some hair follicle types. In this regard, it is of note that while scalp hair follicles gray early, hair follicles of the eyebrow and eyelash gray slowly indeed even in the same individual. This may be related to differences in ROS handling apparatus in these different hair follicle types. For example, while scalp hair bulb melanocytes express undetectable levels of dopachrome tautomerase, similar melanocytes in eyebrow follicles express this melanogenic and oxidative stress-protective enzyme [10, 48].

As mentioned above, the hair follicle pigmentary unit is maximally functioning during post-adolescence and early adulthood when terminal hair growth is optimal and hair color has settled to its preferred tonal variant, i.e., when fully responsive to the postpuberty hormonal stimulus. This age group corresponds to a scalp follicular melanin unit a few hair growth cycles old. Given an average hair cycle length of 3.5 years, some individual scalp hair follicles will experience fewer than ten melanocyte re-seedings from the presumptive reservoir in the average "gray-free" lifespan of 35–40 years for Caucasians [22] (Fig. 9.3).

The onset and progression of graying or canities correlates closely with chronological aging (but not with photoaging) and occurs to varying degrees in all individuals eventually, regardless of gender or race. The age of onset is genetically controlled and inheritable, such that on average Caucasians begin to gray in their mid-30s; Asians in their late-30s; and Africans latest in their mid-40s. Indeed, hair is said to gray prematurely only if it occurs before the age of 20 years in whites, before 25 years in Asians, and before 30 years in Africans. Although not formally tested, a good rule of thumb is that by 50 years, 50% of people have 50% gray hair [57]. Even the term "gray" can be controversial for some, in that as a color may be illusory rather than real, i.e., the impression of gray is provided by the admixture of fully white and fully pigmented hair. The implication here is that unpigmented hair emerges from the surface of the epidermis already as a white hair fiber (Fig. 9.4) or that the hairs are demarcated as two-tone rather than

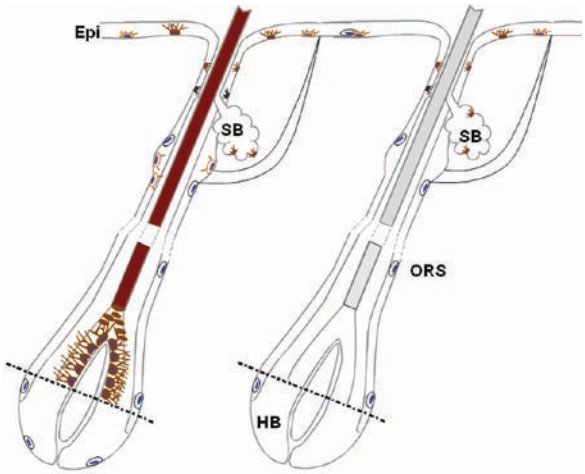

Fig. 9.3 Cartoon of pigmented and canities-affected human anagen scalp hair follicle, showing loss of melanization in the hair bulb and hair shaft with graying. Note the continued presence of few amelanotic melanocytes in the outer root sheath (ORS) and in the most proximal and peripheral hair bulb (HB). *SB* sebaceous gland and *Epi* epidermis (cartoon courtesy of Dr EMJ Peters)

with color dilution. However, this author has often observed canities to affect individual hair follicles during a single anagen VI growth phase, such that there is a gradual loss of pigment along the same hair shaft. There is more to our perception of gray/white hair than just an absence of melanin. Moreover, the hair fiber is not only a complex interplay of many physical characteristics of color but also hair fiber geometry and curvature that can determine shine and luster. Thus, the perception of graying can vary significantly in the general population with early graying being first noticeable in dark-haired individuals. Paradoxically, however, graying can be more extensive in these dark-haired individuals before reaching the blanched effect; the reverse is true for blond hair. The rate of graying is also highly variable not only on different areas of the scalp but also across the body. This may reflect variations in original melanocyte precursor seedings during melanoblast migrations in embryogenesis. Thus, scalp hair graying first appears usually at the temples, and spreads to the vertex and then the remainder of the scalp, affecting the occiput last. Beard and body hair are usually affected later.

Hair bulb melanocytes and precortical keratinocyte are the interactive elements of the follicular melanin unit [53, 51, 57]. There is currently much interest in dissecting the nature of this interaction, with in vitro evidence from mixed origin co-cultures that the

Fig. 9.4 Unpigmented human anagen scalp hair follicle showing loss of melanization in the hair bulb and hair shaft. Note that the lack of melanization in the bulb increases the visibility of the peri-follicular vasculature

keratinocyte directs the melanocyte to produce the type of melanin matching the keratinocyte donor's skin phototype [67]. Moreover, melanin transfer to the keratinocyte partner cells appears to reduce the latter's proliferative potential and rather may stimulate their terminal differentiation. Thus, it is perhaps to be expected that precortical keratinocyte behavior may change in the absence of melanocyte influence. There is some clinical evidence for this: white beard hair appears to grow faster than adjacent pigmented hair *in vivo* [27] and white hair follicles exhibit a higher rate of hair fiber elongation *in vitro* than do matched pigmented hair follicles [2]. In this context, melanosomes may therefore act as regulatory granules [39], for example, by providing a buffer for calcium with implications for second messenger/cell signaling in melanogenesis, melanosome transfer, and keratinocyte differentiation. Furthermore, the saturation binding of transition metals (e.g., iron and copper) to melanin is also likely to influence the antioxidant defense of the melanosome-receiving keratinocyte [64]. Moreover, melanocytes as producers of a range of bio-response modifiers (e.g., cytokines, growth factors, eicosanoids, adhesion molecules, and extracellular matrix) can influence the behavior of neighboring keratinocytes [47].

Evidence of melanocyte–keratinocyte interactivity can also be perceived clinically in the hair fiber too, with graying hair commonly coarser, wirier, and more unmanageable than its pigmented equivalent. Here too, the absence of melanin reflects a change in the chemical and physical properties of the postpigmented hair fiber [62]. Additionally, gray hair is often unable to hold a set and is more resistant to incorporating artificial color. These changes have significant implications for the cosmetics industry. On the basis of the above findings, it appears that graying hair follicles may re-program their matrix keratinocytes to increase the production of medullary, rather than precortical, keratinocytes.

9.10 Conclusion

We are now entering an exciting period in human hair pigmentation research, characterized by a gradual shift from over-reliance on the murine systems. One particular fruitful topic for the study may be to elucidate the function of the amelanotic melanocytes distributed in the outer root sheath of human scalp hair follicles. It will be important to determine if these cells are indeed progeny of the so-called stem cells from the bulge area of the hair follicle, and if so, whether they retain some stem cell characteristics themselves in vivo. Clinical evidence suggests that some of these immature outer root sheath melanocytes may also be available for repigmentation of both the hair follicle itself, but perhaps more importantly clinically, the epidermis especially after wounding [44]. Alternatively, they may represent a subpopulation of transient or migrating melanocytes that can only differentiate when in a permissive microenvironment, e.g., melanogenic zone close to the follicular dermal papilla. The reversal of canities after some types of therapy, e.g., radiation/drug may involve a cytokine-induced activation of outer root sheath melanocytes. It should be considered whether these occurrences may instead involve true stem cell melanocytes in the bulge. A second prong of attack should be devoted to slowing or correcting the deficit in the existing melanocyte populations, i.e., prophylactically, before the melanogenic zone is fully depleted.

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10 Oxidative Stress Associated Melanocyte-Loss and Hair Growth Capacity in the Aging Hair Follicle: A Model for Tissue-Specific Aging

Eva M. J. Peters

Core Messages

- Melanocytes are continuously lost from the aging hair follicle resulting in the obvious graying (canities) that visibly marks our steady decline.
- Many roads appear to lead to this Rome either involved in the regular aging process, or causing premature Canities. Among them, oxidative stress and an altered peri- and intrafollicular neuroendocrine milleu appear to be key mechanisms enabling or forcing melanocytes to respond to stressors ranging from exogenous oxidative stress via inflammatory stress to psychoemotional stress in a highly selective and sensitive fashion.
- However, growth of aging hair follicles – at least in the beginning of the process – is not affected by graying. The hair follicle melanocyte therefore appears to be a kind of sentinel indicating ongoing aging, and the presence of stressors leads to a premature decline of the hair follicle pigmentary system on the one hand, but protection from oncogenic DNA damage on the other hand.

10.1 Matching the Aging and the Graying Process: Common Pathways of Steady Decline or Easy Way Out?

Graying is an enigmatic sign of aging indicating our steady decline and putative loss of power. In our vain society, even finding a new job or spouse may depend on a nicely coloured head [18]. Especially to premature graying, we find many references in the scientific and belletrist literature claiming it to be induced by psychoemotional stressors and our lack of capacity to cope with it. However, we find ourselves frequently surprised by the observation that an early gray head may top a well-kept and young-looking brain and body. Hence, deep down in the hair follicle the loss of actively pigment-producing and transferring melanocytes from the growing hair follicle may mark a strategy to cope with environmental and endogenous stressors imposed on our skin and hair follicles during every minute of our lives [18, 28].

Among these stressors, oxidative stress is highly common [5, 89]. In the hair follicle, melanocyte oxidative stress is high owing to the continuous production of melanin over a long period of time, e.g., one growth phase - anagen - lasts many years in humans (Fig. 10.1). This involves enzymatic processes generating, for example, H_2O_2. It does, therefore, not surprise that the hair follicle melanocyte is well equipped for oxidative stress defense. However, owing to aging or exposure to additional oxidative stress, e.g., UV-exposure, inflammatory stress, direct exposure to exogenous oxygen radicals and their comrades, or even psychoemotional stress, melanocytes easily loose their balance and acquire oxidative stress damage, ultimately leading to their apoptosis [28, 15, 36, 37, 44, 83].

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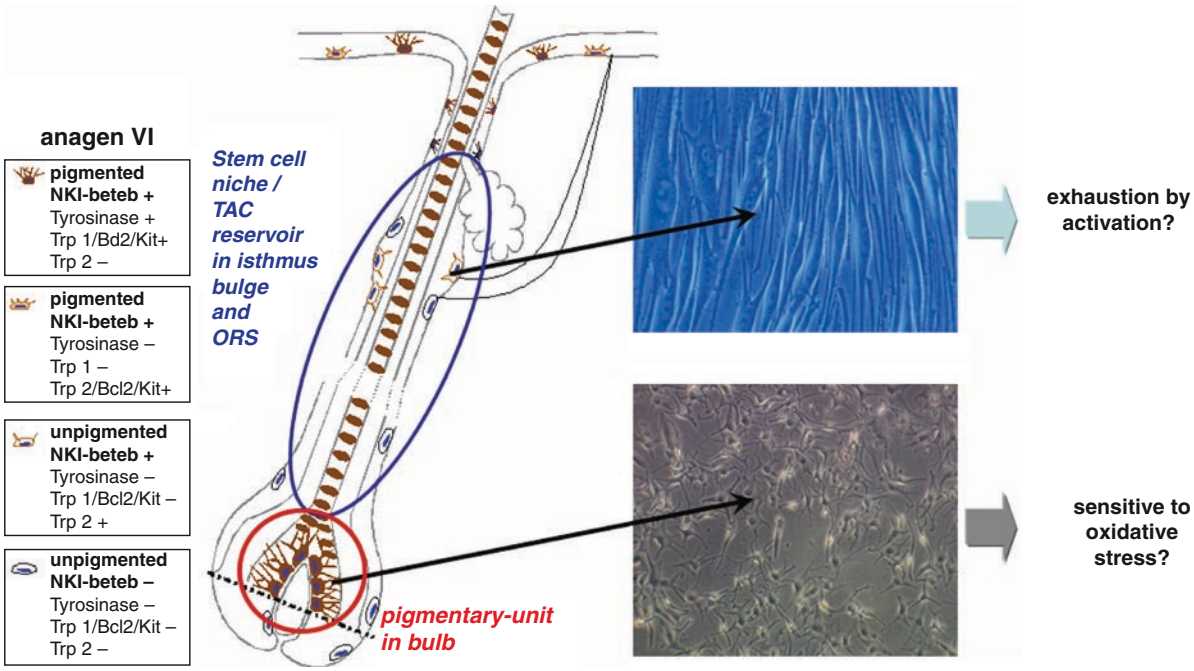

Fig. 10.1 Regeneration of the hair follicle pigmentary unit during the aging process. This schematic drawing depicts location, differentiation, and fate of the different melanocyte subpopulations during the aging process. *Trp* tyrosinase-related peptide

On the one hand, in the wake of these events graying is commonly observed, for example, after excessive UV-exposure [22, 44], toxic insults [56, 62], freeze-branding [46], in chronic inflammatory skin diseases such as alopecia areata [2, 50] or atopic dermatitis [26], and other inflammatory situations such as in the context of melanoma defense. These events cause hair follicle melanocytes to adapt to apoptosis not unlike the events observed during the termination of the hair cycle – catagen (Fig. 10.1). Even the often described but only once documented overnight graying after major life events may be understood along this line of thought [24, 61].

On the other hand, sudden re- or hyperpigmentation of hair is now and again reported after brief irradiation, acute inflammation, or recovery from chemical damage. These "stressors" appear to act rather like the melanocyte growth promoters that aid reestablishment of the hair follicle pigmentary unit during hair growth induction – early anagen (Figs. 10.1 and 10.2). Accordingly, UV and laser light dose dependently increase the pigmentation of cultured melanocytes [1, 14]. Moreover, melanocytes have repeatedly been shown to respond to a certain amount of damage such as telomere disruption or DNA damage by enhanced proliferation, differentiation, and sometimes misplaced pigmentation [6, 27, 35]. Hence, damage of melanocytes by, e.g., oxidative stress may either kill or activate melanocytes possibly in a dose-dependent fashion. One could hypothesize "a little stressor a day, keeps the hair dye away." However, a stress that strains the melanocyte's coping strategies simply represents an overdose and induces premature differentiation and subsequent loss of melanocytes from the actively pigment-producing cell pool [1, 5, 35].

One of the biggest challenges in hair follicle and aging research is therefore to define what exactly comprises a supportive and regenerative – and may be sometimes even challenging environment – to promote the function and growth of precious and sensitive cell populations such as the hair follicle melanocyte. Such an environment would maintain a healthy skin, in general, and a productive hair follicle melanocyte, in particular, rather than promote its premature differentiation and subsequent elimination.

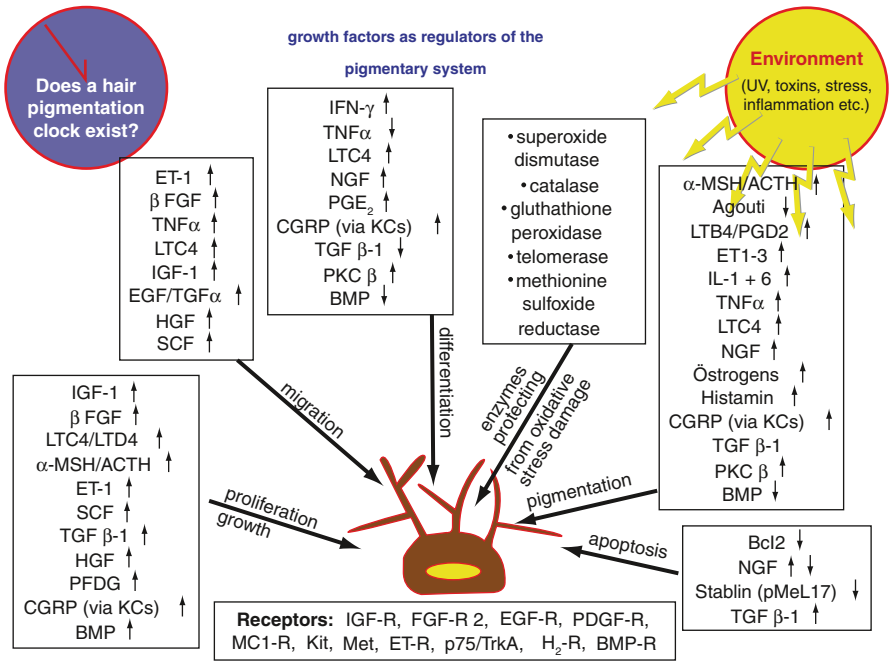

Fig. 10.2 Endogenous ("hair cycle clock") and exogenous regulators of melanocyte's lifetime. Hair follicle melanocytes and their amelanotic precursors are highly specialized cells. Besides proliferation and apoptosis, their life cycle is regulated by a complex differentiation process, the ability to migrate to their designated site of action, and their specific synthesizing activity, melanogenesis. This figure summarizes factors known to date that regulate distinct processes of melanocyte activity. Most of these regulatory mechanisms, however, have been studied in cultured epidermal melanocytes and their role in the hair follicle melanocyte remains to be determined. Regulators written in blue have been reported as altered in aging individuals. Changes in the expression or synthesis of any of the factors identified in the figure can lead to disturbances in downstream signaling processes and ultimately in disturbed pigment production and transfer to the target keratinocytes. Analyzing the role of this wide variety of growth factors and the associated signaling cascades in the aging process of the hair follicle and probing their potential as therapeutic targets for anti-graying agents offer themselves the prime research targets in the analysis of the graying process. *ACTH* adrenocorticotropic hormone; *Bcl* B-cell CLL/lymphoma 2; *bFGF* basic fibroblast growth factor; *BMP* bone morphogenesis protein; *CGRP* calcitonin-gene related peptide; *EGF* epidermal growth factor; *EDN* endothelin; *HGF* hepatocyte growth factor; *IL* interleukin; *IFN* interferon; *IGF* insulin-like growth factor; *KIT* v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *LC* leukotriene; *MC* melanocortin; *MSH* melanocyte stimulating hormone; *NGF* nerve growth factor; *PDGF* platelet-derived growth factor; *PG* prostaglandin; *R* receptor; *SCF* stem cell factor; *TGF* transforming growth factor; *TNF* tumor necrosis factor; *Met* met proto-oncogene (hepatocyte growth factor receptor); *Trk* tyrosine kinase

10.2 Decline of a Supportive Growth Factor Milieu

One important factor certainly involved in the decline of actively pigment producing melanocytes in the aging hair follicle is the decline of growth factors supporting hair follicle melanocytes. Growth factors such as stem cell factor (SCF) or nerve growth factor (NGF) are produced less in the aging skin (Fig. 10.2). In addition, the expression of their receptors can be changed and result in enhanced apoptosis rather than proliferation and pigmentation [81, 84].

An increasing number of growth factors and neuronal signaling molecules such as transforming growth factor beta (TGFβ), NGF, and substance P (SP) are shown to be involved in the timing of the hair cycle [25, 67, 68], i.e., in anagen-catagen transformation. These factors are also known to directly or indirectly regulate epidermal melanocyte functions (Fig. 10.2) as well as cutaneous immune functions. Moreover, their expression is altered in the aging and the transformed melanocyte [49, 54, 81]. Because of the well-characterized roles of these factors in psychological and physical stress responses such as stress-associated immunomodulation [4, 12], they

may also be involved in stress-associated premature graying [5, 44].

TGFβ is upregulated in the hair bulb during catagen [25] and has been shown to antagonize the growth promoting effects of NGF on melanocytes [71, 76]. Physical and psychological stress have also been shown to induce TGFβ expression [3, 44, 47]. Interestingly, TGFβ is commonly appreciated as an anti-inflammatory agent but recruits leukocytes, a major source for oxidative stress in inflamed tissues, to the skin [19]. Also, TGFβ mediates apoptosis through oxidative stress, downregulation of Bcl-molecules, and disintegration of the mitochondrial respiratory chain in many cells types [33, 41, 72]. This makes TGFβ a prominent candidate in the induction of premature canities, elegantly linking emotional and oxidative stress to the apoptosis of differentiated melanocytes.

SP is probably the best investigated psychological and physical stress-associated hair growth modulator. Also interesting in the context of perceived stress-related alterations is that CRH has been shown to be downregulated during chronic perceived stress [38], an effect that is dependent on substance P [16]. This leads to a reduction in ACTH production and subsequently α-melanocyte stimulating hormone (αMSH). Both of these factors are critical components for pigment production [13, 80, 86]. αMSH is additionally an important immunosuppressor [45], which regulates anti-inflammatory T-cell function [79] and acts to reduce oxidative stress [32].

The actions of NGF in hair follicle melanocyte biology appear to be Janus-headed, as it has been shown to either promote melanocyte proliferation or apoptosis dependent on the expression of its high- and low-affinity receptors TrkA and p75 or presence of its precursor molecule pro-NGF [70, 79] NGF has also been shown to be expressed in hair follicles in a hair cycle-dependent manner and in increased levels during the stress-induced premature transition from anagen to catagen [65]. Also, its growth promoting effects on melanocytes are antagonized by TGFβ. This makes NGF another candidate linking different modalities of stress to melanocyte apoptosis.

Interestingly, if skin is constantly exposed to a growth factor cocktail that supports melanocyte proliferation and differentiation – a suggestive strategy for anti-aging therapy – we are confronted with a highly unpleased development: melanoma formation [7]. Here, TGFβ appears to be one of the players and promotes melanocytede differentiation and proliferation of precursors [48], which plays a role in melanoma metastasis. Moreover, melanomas may contain cancer stem cells derived from activated melanocyte stem cells [11], though loss of melanocyte stem cells due to premature differentiation appears to be one of the reasons for graying after irradiation or exogenous oxidative stress [35]. Thus, strategies aiming at replacement of an "aging-milieu" with a "eternal-youth" milieu have to be carefully evaluated for potential malignant developments.

Taken together, melanocytes are equipped with receptors for many stress mediators and inflammatory signals, and respond to a wide variety of signaling molecules, which may be viewed as adjuvants in the cutanous stress response such as acetylcholine, calcitonin gene related peptide, αMSH, NGF, interleukin 1, tumor necrosis factor α, etc. [29, 43, 66, 85, 90] (Fig. 10.2). In this context, an altered growth factor milieu may not only be induced by the aging process but also be part of an alarm system during the cutaneous stress response, ultimately leading to premature loss of pigmentation in hair follicles through lack of support for differentiated melanocytes and enhanced turnover of precursors (see below) while protecting from malignant development.

10.3 Anti-Oxidative Defense of the Aging Hair Follicle

In graying human hair follicles, it is a long-standing observation that the remaining melanocytes often exhibit vacuolation as indicators of oxidative stress and upcoming apoptosis [61] Similar to the changes observed during catagen development in mice [82] or in inflamed and physically or toxically stressed skin [50, 60, 63], melanocytes become round and fat with few and swollen dendrites and clumps of melanin granules intra- and extracellularly. These intriguing morphological observations were the first to suggest oxidative stress as one cause of melanocyte death during aging.

The endogenous antioxidant and anti-apoptotic agent Bcl-2 links exogenous growth factor and biological stress signals to intracellular oxidative stress and premature graying. It is highly expressed in melanocytes and is located in the mitochondrial membrane,

where it prevents melanocyte apoptosis by reducing free radical levels in the cell. Most intriguingly, low levels of this factor in Bcl-2-knockout mice lead to premature graying in these animals [34, 91], and graying hair follicles show a diminished to absent expression of Bcl-2 in humans.

Other oxidative stress-defense mechanisms include high catalase, superoxide dismutase, glutathione peroxidase, and methionine sulfoxide reductase, which are found in high levels in differentiated melanocytes and may also be relevantly altered during the aging process [64, 69]. A stress load, for example, rises to extremes such as induced by cold or heat, oxidative bleachers, repeated excessive psychosocial stress, or other factors, or when melanocytes lose the capacity to cope with the oxidative stress owing to mitochondrial deletion or decreased levels and function of antioxidant enzymes, and contain oxidized enzymes no longer functional in melanogenesis such as tyrosinase. The system may then lose its balance and the damaged melanocytes ultimately [23, 36, 77, 81, 87, 89].

Intriguingly, melanoma cells, the skin tumor cells with the earliest onset and highest tendency to fatal metastasis, show high oxidative stress-defense mechanisms, the less differentiated and more malignant they are [51, 52, 69]. Over-activated oxidative stress-defense mechanisms may thus represent one strategy of melanoma cells to escape endogenous control mechanism eliminating damaged cells from the system. From this perspective, the easy death of melanocytes on any kind of stress, especially after exposure to the mutagenic power of oxidative stress, may mark their role as sentinels in the skins' protection from damaging insults and premature aging or even tumor development.

10.4 Selective Mitochondrial Deletion and Aging of the Hair Follicle Pigmentary Unit

One of the more important targets for oxidative damage is DNA. In comparison with the nucleus, mitochondria have a less effective DNA repair ability, so DNA rearrangements could appear and accumulate significantly more frequently in this organelle. This makes mutations of the mitochondrial genome an ideal marker for cellular oxidative stress. The discovery that the incidence and degree of mitochondrial DNA damage is closely related to the age of the organism over 30 years ago led to the development of the "mitochondrial theory of aging" [30, 31, 53].

In essence, this theory proposes that, through ROS-caused mitochondrial DNA damage, the efficiency and function of the respiration chain is upset leading to a buildup of electrons in the mitochondrion. These free electrons react with oxygen to produce more ROS and further DNA damage – thus creating a positive feedback loop. Additionally, it has been found that, through the compensatory increase in the damaged and less productive mitochondria, there exists, during the course of cell division, a selective advantage for the mutant DNA over time. Subsequently, the accumulation of nucleic DNA damage in the course of the aging process has been shown to correlate with the level of mitochondrial DNA deletions [9, 88]. Together, these observations lead to the formulation of the "free radical theory of aging," a theory that has had a major impact not only on scientific concepts of aging but also on dieting concepts, cosmetics production, and general ideas about healthy life styles in our society.

The presence of mitochondrial deletions has been used as markers for cellular oxidative stress in a number of tissues, including hair follicles in smokers [42] In the light of the selectively high oxidative stress in the hair follicle melanocyte, detection of mitochondrial DNA mutations in the aging hair follicle appeared a useful tool to us to investigate the role of oxidative stress in premature melanocyte apoptosis. We were quite recently able to demonstrate, that the hair follicle melanocyte is among the first cells in the skin to acquire mitochondrial DNA deletions with their premature death (Fig. 10.3) and subsequent visible graying as a result [5]. This process involves a decline in Bcl2 expression in melanocytes in graying hair follicles indicating an ineffective protection from oxidative stress during the aging process. Thus, we proposed a "mitochondrial theory of graying," where endogenous oxidative stress leads to steady DNA decline selectively in the hair follicle melanocytes with their subsequent loss from the pigmentary system.

Others have shown that UVB may contribute to the generation of common deletions in the hair follicle [40, 44], and melanocyte apoptosis may be accelerated by other DNA-damage events such as altered telomeres during the aging process [5, 21, 23, 83] demonstrating that this process may be speed up on the exposure to exogenous stressors generating oxidative stress and DNA damage.

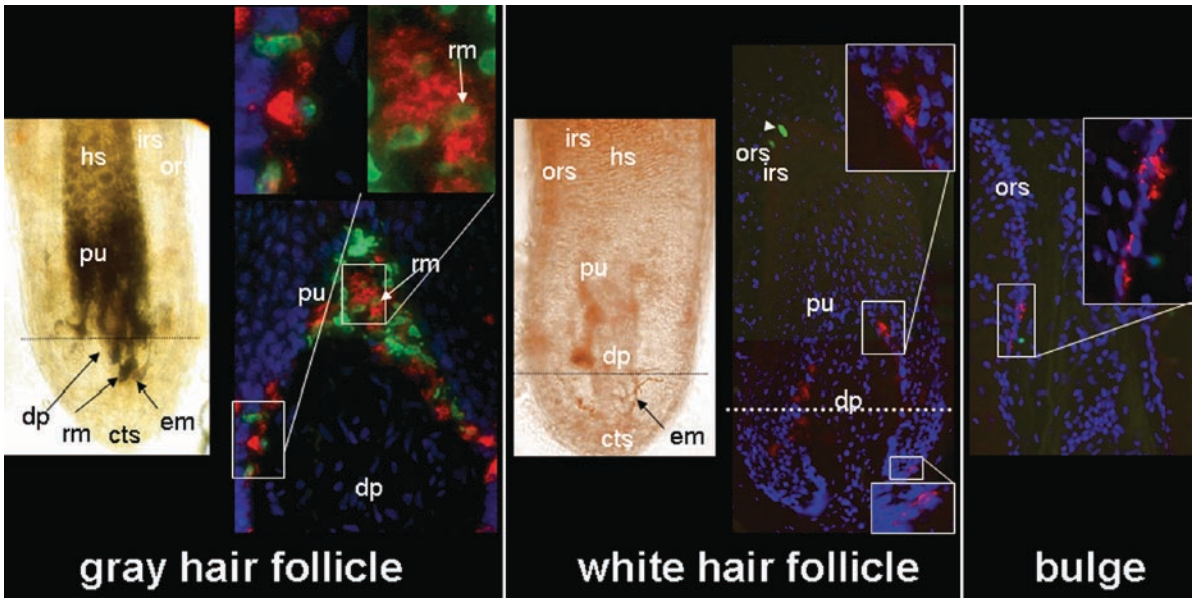

Fig. 10.3 Melanocytes persist in the graying hair follicle's outer root sheath and bulge were they show ectopic differentiation. *pu* pigmentary-unit; *hs* hair shaft; *irs* inner root sheath; *ors* outer root sheath; *dp* dermal papilla; *rm* rounded melanocyte; *em* ectopically differentiated melanocyte; *cts* connective tissue sheath. *Red label*: NKI-beteb (pan-melanocyte marker), *green label*: TUNEL (apoptotic nuclei), *blue label* - Dapi (nuclei). *Boxes* indicate magnification

10.5 Survival of the Melanocyte Stem Cell Pool in the Hair Follicle

Together, a nonsupportive growth factor milieu, a decline in oxidative stress-defense mechanisms, and increased DNA damage are factors suited for the stepwise elimination of the actively pigment-producing melanocyte population in the hair follicle during the aging process (Fig. 10.4). But should these not be replaced by melanocytes generated from the stem cells residing in the isthmus and bulge region of the hair follicle [55, 59, 75]? Here, a population of nonpigmented melanocytes was shown to remain detectable even in the gray hair follicle (Fig. 10.3), a while before the discovery of melanocytic stem cells in this location [58, 64, 82]. However, following the differentiated melanocyte pool in the pigmentary unit during progression of graying, melanocytes are continuously lost from the stem cell niche [17].

Despite their documented progressive loss during the aging process, the melanocyte stem cell population in the outer root sheath of the hair follicle must have some regenerative capacity. Repigmentation, for example, has occasionally been shown to occur within the same hair growth phase [81]. Also, even gray and white hair follicles still pose some melanocyte precursor cells in their outer root sheath [5], though it may take electron microscopy to detect them [64]. A regeneration of the pigmentary unit after damage is therefore possible and can occur within the anagen hair follicle without being transgressing through regression and early anagen to rebuild a functioning pigmentary unit.

Recent observations now suggest that it is time to break with yet another dogma in hair follicle biology. Years back, exhaustion of the hair follicle melanocyte stem cell pool by enhanced turnover of melanocytes on challenge and exhaustive proliferation of melanocyte precursors in the isthmus and bulge region was denied. Stem cells were considered as inexhaustible as any other stem cell population. Long since, stem cell decline has been recognized as an important mechanism of aging and it does not surprise that this is now also realized in hair research [35]. This process involves premature differentiation of melanocytes in the stem cell niche of the hair follicle, e.g., after exposure to H_2O_2 [35] – apparently, a process under the control of Ataxia-telangiectasia muted (ATM).

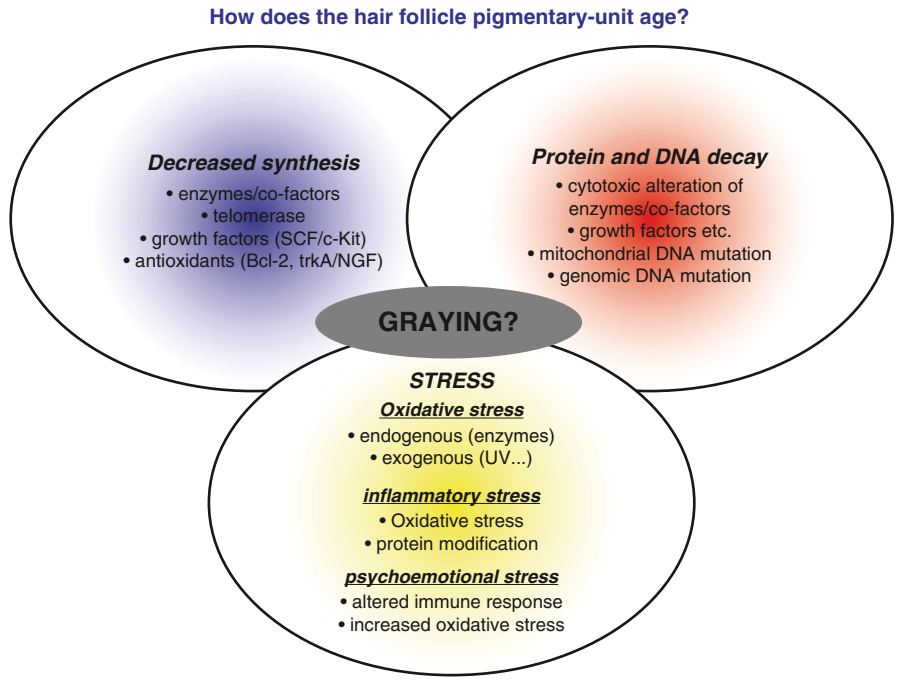

Fig. 10.4 Hypothetical scenario: Pathways interlinking in hair graying. Abbreviations: see Fig. 10.2

Thus, enhanced oxidative stress damage promotes compensatory mobilization of melanocyte stem cells to replace the loss of functional melanocytes in the pigmentary unit. However, this process exhausts the hair follicle's capacity to renew.

10.6 The Role of Psychoemotional Stress for the Graying Process

Each and every review on graying and many interviews with the experts in the field suggest psychoemotional stress to be involved in the above mechanism and clinically contribute to the development of gray hair. Pathogenetic explanations for premature graying (canities) and particularly its connection with emotional or physical stress are, however, more than sparse. Future experiments will hopefully support the hypothesis we can generate from our knowledge of melanocyte biology, on the one hand, and the ways of the cutaneous stress response, on the other hand. Besides the constant challenge to the integrity of melanocytes in the pigmentary unit of the hair follicle by oxidative stress [8, 39, 57] additional oxidative stress, e.g., as caused by inflammatory infiltrates [2, 25] can act to accelerate the decline in the anti-oxidative ability of the melanocyte. In this context, it is plausible that immunomodulatory effects of emotional and physical stress [78] and subsequent increased oxidative stress may exist.

10.7 Finding Ways Out of the Stress

Protection from oxidative stress through different channels has so far proven to be the most effective approach to reduce graying and rescue the cutaneous aging process [19]. Under physiological conditions, e.g., the neurotrophin NGF can rescue melanocytes from apoptosis via induction of Bcl-2 [73, 92]. Protecting hair follicle melanocytes from oxidative stress by the application of superoxide dismutase was shown to prevent graying [22] and L-methionine through prevention of Met oxidation should also be able to prevent stress-induced cellular and genetic damage and hair graying [89]. In addition, application of placental sphingolipids may protect from graying [74].

Take Home Pearls

- Melanocytes have a tightly organized oxidative stress-defense apparatus due to the necessity to control endogenously generated oxidative stress and their exposed position in the skin.
- Upon oxidative stress challenge, their defense holds to a certain degree, than rapidly breaks long before the neighboring cell populations respond.
- On the one hand, enhanced apoptosis and loss of functioning melanocytes from the pigmentary unit is the result; on the other hand, melanocyte stem cells in the niche become activated.
- The resulting enhanced turnover diminishes the melanocyte stem cell pool.
- Any stress contributing to oxidative stress may induce the above changes ranging from radiation via inflammation to even emotional stress (Fig. 10.4).

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11 Human Hair Follicle Melanocytes as a Proxy Cell Type in Neurodegeneration Research

Nikolaos Papageorgiou, Elisabeth Carpenter, and Desmond J. Tobin

Core Messages

- The skin has enormous capacity to act as a peripheral neuroendocrine organ where it can play a major role in maintaining a constant internal body environment or homeostasis. This is perhaps not too surprising given the skin's strategic location between the external and internal environments.
- The continuing extension of human longevity has focused much attention on the elucidation of mechanisms of skin aging, including the contributions of intrinsic (e.g., genetics, evolutionary selective pressures) and extrinsic (e.g., environmental insults and stress) factors to this.
- Melanocyte aging is of interest not only for its implications in skin homeostasis, but also for hearing, vision, and as a model system for physiologic and pathologic neuronal cell aging, e.g., Alzheimer's disease.
- In this chapter, we review the current knowledge on the potential for neural crest-derived melanocytes of the hair follicle (given their particular vulnerability to aging via canities) as a proxy cell type for the elucidation of neurodegenerative change targeting neural and neuronal cells.

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11.1 Introduction

One of the great challenges of modern medicine and for social care has been improving the quality of our lives as we age. However, our successful extension of average life-span has unfortunately come at the cost of a proportional increase in the incidence of age-related neurodegenerative disorders, including the dementias. Predominant amongst these is Alzheimer's disease [70] and motor-neuron impairment (ataxic) disorders such as Parkinson's disease, Huntington's chorea, and amyotrophic lateral sclerosis (so-called Lou Gehrig's disease). In the case of dementia, it is estimated that over 25 million people worldwide are affected, resulting in an enormous and unremitting socioeconomic burden [28]. However, addressing strategies for study of neurodegenerative disorders is not without its intrinsic difficulties; this is evidenced by Alzheimer's disease identified over 100 years ago and yet despite attracting enormous research activity and funding has remained obstinately impenetrable to definitive therapeutic applications.

11.2 Current Neuronal Models

Research directed at unravelling neurodegenerative disorders has been hampered by the significant limitations of *in vitro* models that can simulate the cellular status of human neurons of the brain and the microenvironments in which they normally function. Given the inaccessibility of central neurons, current research in neurodegenerative disorders relies to a great extent on the use of neuronal cell models. Such *in vitro* models can be divided into two categories: (a) transformed cell lines that, under certain conditions, can present with

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Table 11.1 Typical cell types used as neuronal models in neurodegenerative disorders research

Cell type	Derivation
PC12	Pheochromocytoma (adrenal medulla) – rat
Neuro2a	Neuroblastoma – mouse
COS7	Immortalized fibroblasts from African monkey kidney (C. aethiops)
SH-SY5Y	Neuroblastoma – human. Third generation, i.e., cloned from SH-SY5, which is from SH-SY, which is from SK-N-SH
H4	Neuroglioma – human. With complex aberrant karyotype

neuron-like properties and (b) primary cultures of neuronal cells, isolated from animal tissue (Table 11.1).

Common transformed cell lines include rat pheochromocytoma (PC12) cells, derived from a tumor of the adrenal medulla [30]. Following incubation with nerve growth factor (NGF) for 24 h, PC12 cells stop dividing and adopt a phenotype resembling peripheral neurons, which includes the outgrowth of neurites [88]. For research focusing on the properties of central neurons, one cell line frequently used is murine neuroblastoma cells (Neuro2a) that can be induced to sprout neurites after incubation with retinoic acid [71]. Generally, transformed lines are preferred by workers in the neurodegeneration research field because of their capacity to provide very high cell yields and, subsequently, large amounts of protein and genetic material for experimentation. Nonetheless, despite their convenience, transformed cells differ significantly from their primary post-mitotic neuronal counterparts, thus stimulating the need to develop neuronal primary cultures [40]. The most commonly used primary neurons are hippocampal [21, 24] and striatal in origin [32, 90], isolated from embryonic and newborn rats and mice. Other primary neuron cultures include retinal ganglion neurons [7], dorsal root ganglion neurons [12], and superior cervical ganglion neurons [31].

11.3 Difficulties with Current Neuronal Models

However, all the above primary cell types and most of the transformed lines originate from nonhuman animal tissue, and so may not always yield data that realistically represent human conditions. Furthermore, transformed lines such as the PC12 cell line present with high variability, which can frequently result in loss of the original *in vivo* phenotype [78]. Similarly, primary neuronal cultures may be compromised due to damage to their functional interactions with other cells and parts of the brain, while their preparation for culture is generally laborious, time-consuming, expensive, and produces relatively low cell/protein yields [40]. An example of this can be found in a commonly used technique developed since the late-1970s, whereby single primary sympathetic neurons from the rat or mouse superior cervical ganglia are compartmentalized into 20× 200 μm-wide tracks scarred onto the surface of a 35-mm culture dish [13, 65]. The technique is thought to simulate the *in vivo* conditions of neuronal development and neurite outgrowth, with the following caveats: (1) animal neuronal cultures may not, as mentioned above, accurately represent human neuronal properties (especially considering a system as multifunctional as the nervous system), (2) they often do not provide sufficient protein yield (usually a maximum of 5–10 μg) [40]. Added to these are the demanding care of primary neurons in culture and the short life-span of successful cultures, which do not generally survive more than 2 weeks.

11.4 Melanocytes as a Neural/Neuronal Cell Model

The above discussion has outlined the need for the development of novel neural/neuronal cell models. In addition, it emphasizes the need for a neuronal cell model that represents human adult neuronal cells adequately while remaining relatively accessible for long-term cell culture with high cell/protein yields. At the same time, this model cell type should not suffer loss of significant primary tissue properties. In seeking such a cell model, various pieces of evidence have led to the proposal of human follicular and epidermal melanocytes as possible candidates. Moreover, establishing human adult melanocyte cultures is a relatively simple process and has the additional advantage that the cells can be cultured contemporaneously from patients beginning to suffer from, or in advancing stages of, a neurodegenerative disorder, which potentially could provide an enormous advantage for both research and clinical diagnostics.

11.5 Similarities Between Melanocytes, Other Neural Cells, and Neurons

Melanocytes share a number of similarities with neuronal cells, including their common embryonic origin in the neural crest and an obvious dendritic morphologic similarity. The latter also reflects similarities in the functions of the two cell types, such as transfer and secretion of vesicles along axons in neurons and melanosome "vesicles" along dendrites to keratinocytes [76]. Further similarities can be found in the use of the amino acid tyrosine by both cell types for the formation of melanin in melanocytes and catecholamines in adrenergic neurons [76]. Both cell types are supported by surrounding "supporting cells," with epidermal and follicular melanocytes associating intimately with keratinocytes, while maintenance of neurons relies on surrounding glia. While melanin pigment production is a hallmark of cutaneous melanocytes, "neuro"-melanin production occurs in neurons of the nigrostriatal pathway [66], via a biosynthetic process broadly similar to melanogenesis in melanocytes. Melanosomal transfer to keratinocytes through melanocyte dendrites [72] can be viewed to parallel synaptosome transfer and release in neurons through their axons. Such similarities may reflect common shared archetypical properties between these two neural crest-derived cells, and so could be advantageous when using melanocytes as a model for neurons.

Locally, melanocytes are long-living and postmitotic neuroectodermal cells of the epidermis that transduce external and/or internal signals/energy into organized regulatory network(s) for the maintenance of cutaneous homeostasis [76]. There is a remarkable "self-similarity" of these networks (including via melanocortins) at the systemic/CNS and local levels – a fascinating biologic fractal of a universal neuroimmunomodulatory principle [81]. In this way, therefore, melanocytes can act as the upper regulatory arm of the skin neuroendocrine system ("neurons" of the skin and hair follicle).

While the UVR-protective role of the pigmentary system in the epidermis is well recognized, its precise value to the hair follicle is less clear [79]. New experimental evidence has only very recently revealed significant additional functionality for these cells, via their production of classical stress neurotransmitters, neuropeptides, and hormones, and susceptibility to neuron-like aging and degeneration, that they act as neuroendocrine regulators on different levels [66, 76, 79]. The melanocyte also provides the neurosciences with an attractive model to examine neural crest-derived cell plasticity; the skin and hair follicle contain melanocytes in all stages of development from true stem cells to melanoblast, differentiated cells, and terminally differentiated cells [35, 59, 79].

Table 11.2 A comparison between melanocytes and neurons

Melanocytes	Neurons
Originate from the neural crest	
Neurotrophin-dependent cell migration, differentiation, and apoptosis	
Similar dendritic morphology	
Similar receptor expression (e.g., cholinergic receptors)	
Similar profiles of neurohormones, neurotransmitters, and neuropeptides	
Supported by keratinocytes	Supported by glial cells
Use tyrosine for end products (eu/pheomelanin)	Use tyrosine for end products (catecholamines)
Melanogenesis (eu/pheomelanins)	Melanogenesis (neuromelanin)
Melanosome transfer and release from dendrites	Synaptosome transfer and release from axon terminals

A comparison of general biologic properties between neurons and melanocytes is given in Table 11.2. In addition to the above, we have recently reported that follicular melanocytes in culture express two major neuron-specific markers, NeuN [58] and βIII-tubulin [41, 42] (Fig. 11.1). Characteristically, the expression of NeuN was found in the nuclear region of the hair follicle melanocytes, while βIII-tubulin expression was restricted to the cytoplasm, correlating with the respective localization and function of the two proteins.

Cutaneous melanocytes, including hair follicle melanocytes, can produce relatively high-yield, long-lived cell cultures without the potential variability of prolonged transformed cell lines in culture [43, 80, 83]. Furthermore, owing to their derivation from human tissue, melanocytes may match closely the properties of human neurons, something that may not always be true for animal cells.

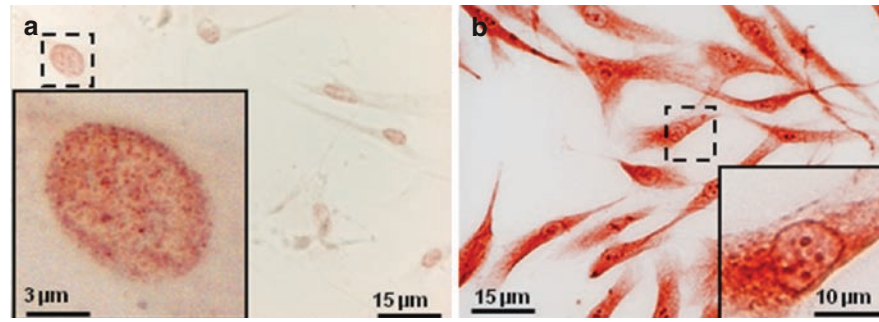

Fig. 11.1 Expression of NeuN (**a**) and βIII-tubulin (**b**) in cultured hair follicle melanocytes

11.6 Age-Related Loss of Pigment in Hair and Skin

Additional support for the use of cutaneous melanocytes as a neuronal cell proxy comes from the appearance of hair whitening, or canities, a characteristic feature of aging in humans [79, 81]. Given the age link between the average onset of canities and the later potential onset of neurodegenerative disorders such as Alzheimer's disease, it is reasonable to suggest that the mechanisms of aging and apoptosis of follicular melanocytes and central neurons may share certain similarities.

For example, "older", more terminally differentiated follicular melanocytes in the hair bulb undergo cell death during the apoptosis-driven catagen phase [84, 85]. By the end of anagen, hair follicle bulb melanocytes begin to retract their dendrites and cease their synthesis of melanin as the activity of key melanogenic enzymes (including tyrosinase, TRP-1) decreases [73, 74, 84, 87]. Notably, the potentially stress-protective dopachrome tautomerase appears to be downregulated upon terminal differentiation at least in scalp hair follicle bulbs [15]. Thus, hair follicle catagen is characterized by a slowing-up of pigment production in bulbar melanocytes, and the subsequent absence of these fully differentiated and highly pigmented melanocytes. It is also thought that during catagen, some melanogenic melanocytes of the anagen bulb undergo apoptosis [84], even resulting in the redistribution of incontinent melanin to the catagen dermal papilla [74, 75, 77, 85]. This view is further supported by the observation that hair follicle melanocytes exhibit TUNEL-positivity during spontaneous and cyclophosphamide-induced catagen in murine hair [84]. For specific discussion on melanocyte loss during canities, please see chapters 1 and 9.

11.7 Melanocyte and Neuronal Cell Apoptosis: Utilization of the C-Kit and P75^{NTR} Receptor Signaling Systems

It is known that the number of epidermal melanocytes decreases by approximately 10% per decade after the age of 30 [14, 64, 82]. Melanin production in the hair follicle decreases with aging during the growth phase anagen where it is thought to be due to apoptosis of differentiated follicular melanocytes. Loss of pigmentation upon the subsequent anagen appears to be related to exhaustion of melanocyte stem cell reserves in the hair follicle bulge [35, 59, 79]. The age-related fall in melanogenic potential is thought to contribute to the pallor of aged skin, which reflects depletion of epidermal melanocytes. However, these age-related changes in the pigmentary system can be subtle [92, 93] and the precise manner by which age-related melanocyte depletion occurs is not yet clear. One suggestion is that this involves decreased signaling of the stem-cell factor (SCF) through the c-kit receptor [5, 11]. It is interesting that the SCF/c-kit system has also been shown to play an important protective role in cortical neurons against both apoptotic and excitotoxic cell death, both of which characterize the pathologies of neurodegenerative disorders [22].

Another view holds that melanocyte age-related apoptosis may be associated with a loss of function of the free radical scavenger Bcl-2 [89]. This is of particular interest when drawing comparisons between melanocytes and neurons because of its upstream association with the neurotrophin receptor p75^{NTR}, which plays a major survival/apoptotic role in neuronal cells [61] and neurodegeneration [19] and also for reactive oxygen species (ROS) handling in hair follicle melanocytes [59]. In addition, p75^{NTR} has been linked to the presence of neurotoxic

beta-amyloid (Aβ) plaques in the extracellular space of neurons in the brain – a major pathological pathway in Alzheimer's disease. One proposed mechanism of Aβ-mediated cytotoxicity involves a $p75^{NTR}$ apoptotic cascade, evidenced by the fact that Alzheimer's disease involves degeneration of cholinergic basal forebrain neurons, which express high levels of the $p75^{NTR}$ receptor [29]. Conversely, expression of another neurotrophin receptor, the tyrosine receptor kinase (trkA), which mediates a survival-signaling pathway, reportedly decreases in Alzheimer's disease patients [56, 57]. Furthermore, it was found that the pro-form of nerve growth factor (NGF), which is also increased in Alzheimer's disease [26, 63], binds $p75^{NTR}$ selectively, and induces neuronal cell death [46]. Additional studies have shown that increased ligand activation of $p75^{NTR}$ results in neuronal cell death in animal models [18, 20, 69]. Finally, it has been shown that neurotrophins can affect Aβ production by regulating the proteolytic processing of its precursor protein, APP, through trkA and $p75^{NTR}$ receptors [16]. The outcome of these studies has been to link neurotrophins and their receptors to the pathology of Alzheimer's disease, and especially to the regulation of Aβ generation.

In 1996, Zhai and colleagues reported that human neonatal foreskin epidermal melanocyte cultures incubated with the 40-amino acid isoform of Aβ (Aβ1–40) resulted in a 90% decrease of cell yield after 3–5 days, when compared with the reversed sequence of the peptide (Aβ40–1), which had no effect on cell viability [96]. The authors also reported that incubation with Aβ resulted in the formation of structures that resembled the amyloid plaques observed in the brains of Alzheimer's disease patients, consisting of a central core of dying cells surrounded by a circumferential cluster of degenerating melanocytes. These data led the authors to suggest, for the first time, neural crest-derived melanocytes as a valuable model for neuronal cells in the context of Alzheimer's disease [96].

Additional work from the same group showed that the $p75^{NTR}$-Aβ apoptotic pathway might operate in these neonatal foreskin melanocytes by showing that these cells could undergo apoptosis through the $p75^{NTR}$ signaling pathway [91]. Specifically, this study reported a threefold increase in the expression of the pro-apoptotic protein Bax in melanocytes incubated with Aβ1–40 when compared with control cells, followed by the observation that the addition of NGF to the cultures appeared to inhibit this process. NGF binds with high affinity to the trkA receptor (Kd = 10^{-11} M) and with low affinity (Kd = 10^{-9} M) to $p75^{NTR}$ [9, 18, 19]. The authors suggested that NGF interferes with the Aβ signaling pathway at the receptor level. Moreover, binding of NGF to both receptors in melanocytes appeared to inhibit the expression of Bax, thus promoting survival of the cell. In contrast, melanocytes expressing only $p75^{NTR}$ did not survive. In conjunction with the morphological effects of Aβ on cultured epidermal melanocytes described above, the conclusion drawn by these authors suggested that Aβ-induced apoptosis in human epidermal melanocytes is mediated through the $p75^{NTR}$ pathway, and can be reversed by the presence of NGF. In a broader context, these findings show that the Aβ-associated apoptosis observed in cultured human neonatal melanocytes involves the same signaling pathway as degenerating central neurons of Alzheimer's disease patients, thus demonstrating a functional similarity between these two cell types.

Interestingly, Yaar and colleagues used human neonatal foreskin epidermal melanocytes in comparison with NIH-3T3 fibroblasts to investigate the properties of the $p75^{NTR}$-Aβ interaction [94]. This study also reported epidermal melanocyte apoptosis following Aβ1–40 incubation, and also took into account the aggregation state of the peptide, which is an important factor in studies that investigate the *in vitro* effects and properties of Aβ [36].

Furthermore, a 2003 study demonstrated the expression of the amyloid precursor protein, APP, in cultured epidermal melanocytes and proposed its involvement in the regulation of dendrite motility and the release of melanin [67]. More recently, we demonstrated the expression of both APP and the Aβ1–40 peptide in cultured adult human melanocytes and showed that Aβ1–40 exerts dose-dependent and cell type-specific cytotoxic effects on these cells [62] (Fig. 11.2) matching previous studies on neuronal models [50] and primary fetal neurons [97]. Thus, our data suggests that adult human cutaneous melanocytes can be affected by Aβ in a manner similar to that of neurons in Alzheimer's disease [17], thus supporting the use of these cells as a model for neurons, at least in the context of Alzheimer's disease.

11.8 Melanocyte and Neuronal Cell Sensitivity to Oxidative Damage

Melanocytes generate ROS via oxidation of tyrosine/DOPA during melanogenesis, and so this process can be viewed as "physiologically cytotoxic" that if not

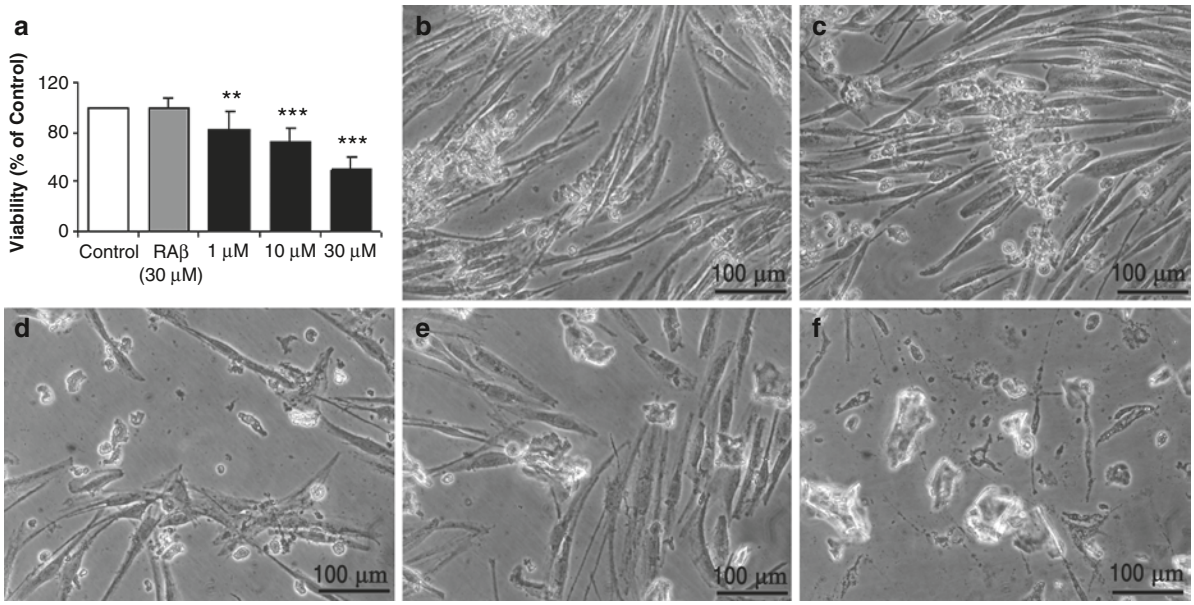

Fig. 11.2 Effect of aggregated Aβ1–40 peptide on elderly human melanocytes. Aβ1–40 effects on melanocytes viability (**a**). Five primary cell cultures tested with each dose in quintuplicate. Compared to the no-peptide and reverse peptide controls, Aβ significantly reduced melanocytes viability at all three doses. (**b**–**f**) Representative phase-contrast views of effects of Aβ1–40 on melanocytes after 72 h; control cells (**b**); reverse peptide (30 μM) (**c**); Aβ1-40 (1 μM) (**d**); Aβ1–40 (10 μM) (**e**); Aβ1–40 (30 μM) (**f**). Bars show mean relative to control data designated 100% + SEM. Key: ** = $P < 0.01$; *** = $P < 0.001$. RAβ; reverse Aβ peptide

sequestered within the melanosome may result in melanocyte damage by breaking of DNA strands, damaging of bases, and DNA–protein cross-linking [4, 5, 33, 87]. It is thought that melanocyte death can ensue via the accumulation of intracellular melanin, despite the ability of eumelanin to exhibit some antioxidant capacity through the sequestration of heavy metals [6]. See chapters 1 and 9 for more on the role of oxidative stress in melanocyte aging during hair graying or canities.

Melanin production may not be a widespread feature of neurons, though this is strikingly evident in the case of neuromelanin in neurons of the nigrostriatal pathway. It is noteworthy that these cells are particularly targeted in Parkinson's disease [25]. However, there is increasing evidence that melanin production can occur throughout the brain and not only in the prominent sites of the leptomeninges and the locus coeruleus (see [66]). Generally, oxidative damage has been implicated in neurodegeneration [27]. The levels of redox-balancing factors in neurons are significantly depleted in neurodegenerative disorders including Alzheimer's disease and Parkinson's disease [37, 49]. Thus, the lack of adequate ROS scavenging may be an initiating signal in neuronal apoptosis in neurodegenerative disorders [34].

The homeostasis of ROS generation is under tight control, comprising a variety of anti-oxidase enzymes (e.g., catalase, superoxide dismutases [SOD], glutathione reductase) and detoxifying molecules (e.g., glutathione, α-tocopherol, carotenoids, ascorbic acid) that neutralize oxidants and maintain them at a level that is safe to the cell. Subsequently, impaired function of these detoxifying factors can lead to compromised antioxidant capacity and so result in the generation of oxidative stress, including destruction of lipids, proteins, and nuclear DNA. These toxic events may then ultimately lead to apoptotic or necrotic cell death [39]. We have recently shown that hair follicle melanocytes cultured from aging skin donors have a compromised antioxidant system when compared with those melanocytes established from younger donors [44, and unpublished]. See chapters 1 and 9 for further relevant discussion.

Similarly, oxidative damage has been implicated in neurodegenerative disorders, and reduced antioxidant capacity has also been shown to cause neuronal cell

death [48]. For example, one study showed that mice homozygous for a targeted mutation in the gene encoding for α-tocopherol transfer protein exhibited delayed-onset (after 12 days of age) retinal degradation and gait abnormalities, arising from an accompanying degeneration of the posterior spinal cord column [95]. To confirm that the absence of functional α-tocopherol transfer protein was solely responsible for the associated behavioral symptoms, the researchers administered the normal protein to mice with the same α-tocopherol mutation, resulting in the prevention of the characteristic pathology. These findings suggest that neurons are susceptible to cell death in the absence of antioxidants, or through mutations of genes encoding antioxidant proteins [45].

Further evidence of a link between oxidative damage and neurodegenerative disorders has come from the discovery of increased transcription of oxidative-handling genes encoding for factors such as catalase, glutathione peroxidase, Cu-SOD, Zn-SOD, and glutathione reductase mRNA in the hippocampus and inferior parietal lobule of brains from Alzheimer's disease patients when compared with healthy controls [2]. In addition, dopaminergic neurons in the substantia nigra from Parkinson's disease patients have been found to exhibit markers of oxidative damage such as lipid peroxidation, protein and nucleic acid oxidation, and toxic products of oxidative damage to lipids like 4-hydroxynonenal [37]. This suggests that there is a strong association between oxidative damage and the onset of neurodegenerative disorders, which describes the current dominant view of researchers in the field [23].

However, given that most of the observations of cell damage have relied on postmortem samples, it is difficult to discern whether the presence of oxidative stress markers in these brains signifies that the disorder itself was initiated and/or promoted by the unbalanced generation of ROS, or if such damage is simply an end-stage phenomenon [45]. Thus, the availability of an accessible model, like hair follicle or epidermal melanocytes, may provide opportunities to probe these cause-and-effect difficulties. The above information provides an understanding of the way oxidative damage is implicated in neurodegeneration, which is a feature of Alzheimer's disease. In comparing melanocytes with neurons, high sensitivity to oxidative damage is an important feature shared by the two cell types, and thus can be investigated through the use of melanocytes as a neuronal model.

11.9 Calcium Homeostasis and Melanocyte and Neuronal Cell Death by Apoptosis

Activation of the trk family of receptors by neurotrophin ligands in neural crest-derived cells can result in the release of Ca^{2+} through the phospholipase-γ – inositol triphosphate/diacylglycerol pathway [10]. Increases in intracellular Ca^{2+} can also occur through the increased activation of the Bax gene, thus resulting in apoptosis [86]. As mentioned above, activation of Bax in melanocytes can be induced following binding of Aβ to $p75^{NTR}$ [91]. Similarly, Ca^{2+} imbalances have been associated with neurodegeneration [52], and these are worth reviewing for the purposes of this discussion on potentially shared processes between melanocytes and neurons.

Ca^{2+} is ubiquitously involved in a wide range of intracellular signals, playing a crucial part in controlling many physiological processes including gene expression, cell differentiation, enzyme activity, exocytosis, and apoptosis. Under normal conditions, the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) ranges between 50 and 200 nM, about 10,000 times lower than the extracellular Ca^{2+} concentration (1–2 mM). This balance is maintained through tight homeostatic mechanisms, which commonly involve the communication between intracellular Ca^{2+} stores and voltage-dependent and ligand-gated ionotropic Ca^{2+} channels in the plasma membrane [8, 53].

Deficient cellular Ca^{2+} homeostasis is thought to be involved in neuronal cell death in neurodegenerative disorders [8, 47, 53]. Postmortem brains from Alzheimer's disease patients have been found to contain increased levels of calpain, a Ca^{2+} release-modulating protein [60], while increased concentrations of bound and free Ca^{2+} have been found in neurons expressing neurofibrillary tangles. Ca^{2+}/calmodulin protein kinase II, an enzyme associated directly with the formation of paired helical filaments [53], is increased in hippocampal neurons, which are prone to Alzheimer's disease-related degeneration [54]. Further evidence that links compromised $[Ca^{2+}]_i$ homeostasis to Alzheimer's disease comes from studies revealing high transglutaminase (a Ca^{2+}-activated enzyme that induces tau protein cross-linking) levels in Alzheimer's disease brains [38, 55]. In addition, cell cultures and animal Alzheimer's disease models

reveal a cause-and-effect connection between affected $[Ca^{2+}]_i$ homeostasis and neurodegeneration. For example, elevated $[Ca^{2+}]_i$ resulting from glutamate receptor overactivation in neurons induces formations of neurofibrillary tangles-like cytoskeletal structures [51].

In melanocytes, Ca^{2+} homeostasis has been associated with melanin production and melanocyte apoptosis [3]. The latter has been paralleled with general Ca^{2+}-driven apoptosis pathways, as described above. It is also notable that low extracellular Ca^{2+} levels have been proposed to promote melanocyte proliferation and melanogenesis, but higher concentrations of extracellular Ca^{2+} only influence the latter [1]. Ca^{2+}-mediated apoptosis in cells, including neurons, may be paralleled in melanocytes. Within the context of the current discussion, it is important to draw comparisons between the two cell types, and especially in the area of cell death, as this is a prominent feature of Alzheimer's disease.

11.10 Conclusion

The above discussion proposes the use of human cutaneous melanocytes (especially hair-follicle-derived) as a proxy for neural and neuronal cells in the context of neurodegeneration studies, particularly Alzheimer's disease research. It has reviewed a number of properties that should be explored to determine the strength of this proposition and provides the framework in which melanocytes must be examined as a potential model for central neurons. Furthermore, an overview of cutaneous melanocyte biology provides a foundation on which it is possible to draw crucial parallels between the two cell types, especially in terms of neural crest-derive cell apoptosis, which is a de facto characteristic of neurodegenerative disorders such as Alzheimer's disease.

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Effect of UV Radiation on Scalp and Hair Growth

12

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Core Message

- Scalp, even covered by hair, is altered by both UVA and UVB irradiations.
- Melatonin is a light-influenced mediator released by the pituitary gland that affects hair biology.
- The speckled subclinical perifollicular melanoderma probably results from the release of melanocortin at the hair follicle openings at the skin surface.
- The mottled subclinical interfollicular melanoderma is related to cumulative Sun exposures.
- Ultraviolet (UV) exposure can lead to early teloptosis (exogen phase) and to the hair eclipse phenomenon (lag phase between two successive hair cycles). The events are responsible for the actinic telogen effluvium.
- Androgenetic alopecia appears to be adversely influenced by UV exposures.
- Actinic field carcinogenesis of the scalp is responsible for most of the actinic keratoses and squamous cell carcinomas of the scalp.
- The hair shaft structure and color are directly altered by UV radiations.

12.1 UV Radiation

Among a wide variety of environmental threats [60], sunlight, in particular its ultraviolet (UV) spectrum, exerts prominent effects on the skin [74]. UV only represents a fraction of the continuous spectrum of electromagnetic radiations emitted by the Sun. They are responsible for many biological effects ranging from acute sunburn to tanning and protracted skin cancers. According to their main effects and their wavelengths, UV radiations are divided into UVC (200–280 nm), UVB (280–320 nm), and UVA (320–400 nm) [81].

UVA radiations from the Sun penetrate the atmosphere and stratosphere ozone layer, while UVB are predominantly absorbed by these layers. As a result, UVA and UVB wavelengths represent 95 and 5%, respectively, of the UV spectrum emitted by the Sun and reaching the Earth's surface. In addition, UVA light is almost not attenuated by clouds and glass. It is emitted at a constant rate in the sunlight from sunrise to sunset. When about 90% of UVB are filtered by the stratum corneum, over 50% of UVA penetrate deeper into the skin, including the papillary and reticular dermis.

The UVC range is highly mutagenic. The fraction originating from the Sun does not reach the Earth's surface because it is absorbed by the stratospheric ozone layer. UVC radiations are also generated by specific artificial light sources, such as arc welding lamps, germicidal lamps, and some lasers. Little is known about low-dose UVC-induced damage on human skin since this kind of exposure is rare. UVC penetration inside the skin is strongly limited. In contrast, UVC cause severe eye irritation and damage [39].

In addition to natural sunlight, many artificial sources including some medical devices emit UVA and/or UVB at doses well above one minimal erythema

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dose (MED). Epidemiological data suggest that such UV overexposures may lead to various skin alterations. In addition, psoralen phototherapy with UVA (PUVA) irradiation results in an increased risk of photodamages that may be more severe than regular UVA or UVB therapies alone [35, 56, 65].

12.2 UV Scalp Weathering

Normal scalp is not fully protected by hair from Sun exposure. Obviously, any hair thinning further declines the natural protection against UV. There are large interindividual differences in the amount of UV light reaching the scalp. For instance, dosimetry assessments have revealed the high daily occupational UV exposure of mountain guides that may exceed 17 MED [42]. Therefore, it is not surprising that both hairy and bald scalps are susceptible to be affected by photosensitive disorders and potentially exhibit signs of chronic photodamages. It is obvious that baldies show more solar elastosis and signs of photocarcinogenesis on the scalp [108]. Some specific disorders named "red scalp syndrome" [94] and "erosive pustular dermatosis" are typically restricted to the light-exposed scalp [6, 69, 99].

Although knowledge of the hair follicle anatomy and cycling dynamics is substantial [34, 41, 48, 78, 88–90], the effects of UV light on the elusive hair clock cycle remain uncertain. Yet the hair follicle appears to be a unique UV bioreceptor [25]. In particular, the anagen phase arrest is influenced by seasons and light exposure in the moulting animal [90].

In Humans, a speckled perifollicular faint melanoderma has suggested the intervention of melanocortins in the vicinity of hair follicles [49, 107]. Acute telogen effluvium may follow solar exposure, particularly in subjects with light pigmented hair [8, 53, 75]. The complex molecular mechanism of androgenetic alopecia [97] may also be negatively influenced by cumulative UV exposure [59, 96]. Conversely, UV exposure is used as a therapeutic agent in some autoimmune hair loss, such as alopecia areata.

12.3 Hair Melatonin Connection

The relative influence of the direct and indirect effects mediated through ocular exposure to intense light, followed by brain stimulation and neuroendocrine messages, remains speculative. Yet, the resulting hair cycle synchronization by light and moulting in animals are in part controlled by endocrine messages possibly involving prolactin and melatonin [3].

Circulating melatonin is mainly secreted by the pituitary gland. It is a chemical messenger of light and darkness and thus acts as a chronobiotic regulator. In addition, melatonin is produced and metabolized by the skin, in particular the upper part of the epidermis, and dermal structures including blood vessels and mast cells, as well hair follicles [30, 84]. It has been demonstrated that melatonin production in human intrafollicular epithelial cells is regulated positively by adrenergic-receptor stimulation [34], but some environmental factors, including actinic radiations, might participate in this regulation. Melatonin affects skin functions through cell surface and possibly nuclear receptors, and has also receptor-independent effects [82]. This molecule has probably a protective role against reactive oxygen species (ROS) and UV-induced damage in the skin [85]. Melatonin participates in the seasonal cyclic activity of hair growth and pigmentation in animals [83]. It is uncertain whether melatonin affects hair growth in Humans. However, topical melatonin applications have been reported beneficial in androgenetic alopecia [13].

12.4 UV Spectrum and the Scalp

UV reactions directly affect the functions of epidermal cells. Many of these effects are mediated by diverse cytokines (IL-10, …), growth factors, neuropeptides, and hormones including the α-melanocyte-stimulating hormone (α-MSH). The latter compound stimulates the melanocyte activity and exerts an anti-inflammatory and immuno-modulatory potential. The melanocortin-1 receptor is also expressed in the epidermis [77].

Since UVB have more intrinsic energy than UVA, they have long been considered to be responsible for most human photodamages. The increased use of UVA sources over the past decades for treating certain dermatoses and for tanning purpose in Sun parlors [64] has revealed the potential cutaneous UVA damage in the course of the daily life [79]. It appears now that many human photolesions are due to UVA in the absence of the alarm signal of sunburn. Therefore, UVA possibly cause greater cumulative alterations, including epidermal hyperplasia associated with photodyskeratotic cells [32], reduction in the density of

Langerhans cells, splitting of the lamina densa [32], discrete inflammation of the dermis with damage of the extracellular damage [31], including disorganization of the elastic fiber network coated by lysozyme [1, 32, 33, 40, 46, 59, 79]. All these features have been observed on the weathering scalp [59].

There is mounting evidence that large doses of UVA produce changes similar to those caused by long-term exposure to solar radiation. A dose of about 20 J/cm^2 for 5 weeks, which corresponds to the regular daily suberythemal UVA dose received by the skin, produces the effects referred here above and, in the long term, causes clinical signs of photoaging [32]. However, these features do not indicate that UVA radiations represent the single cause of scalp photoaging. Indeed, UVB play a substantial role in this process. However, while the role of UVB in the expression of various extracellular matrix components of the dermis is prominent, UVA probably boost these effects.

12.5 Speckled Perifollicular Melanoderma

A faint speckled perifollicular melanoderma is typically seen under UV light illumination on the face and scalp. Such an aspect consists of small melanized spots centered by hair follicles. The so-called ultraviolet light-enhanced visualization (ULEV) method is convenient for revealing these changes [49, 50, 73, 107, 108]. Early subclinical changes in the mosaic pattern of epidermal melanization are particularly prominent in light skinned individuals with a pheomelanin-enriched phenotype [19]. The increased contrast between the subclinical or faint hyperpigmentation and the surrounding skin is the result of the decreased reflection of UV light by collagen because of its absorption by the epidermal melanin.

The speckled perifollicular pattern represents the primary intrinsic melanocytic activation when hair fullness is maximal [49]. The globular pattern is evidenced only when alopecia is present [108]. It corresponds to an accretive process by extension and merging of the perifollicular spots to the interfollicular area.

Aging and chronic exposure to UV light provide a series of signals to the epidermal melanocytic unit [17]. Focal melanotic hypermelanosis occurs where individual melanocytes aggregate and are stimulated to produce more melanin. The melanins are composed of a series of molecules characterized by different levels of polymerization, oxidation, and sulfur content [68]. Melanosome transfer from melanocytes to surrounding keratinocytes is also enhanced under the same stimulation. In addition, chronic UV exposure is responsible for both replicative and stress-induced premature senescence (SIPS) of melanocytes [95], which undergo apoptosis. Decreased epidermal melanization ensues focally. As a result, photoaging, in particular on the scalp, is characterized by a mottled appearance due to an uneven distribution and activity in epidermal melanocytes [19, 50, 73]. The clinical aspect depends on the combination of the individual melanin phenotype, age, cumulative UV exposure, and body site [43, 50].

Several clinical and experimental conditions have highlighted the bicompartmental system in the epidermal melanocyte population. The system indeed corresponds to the perifollicular and the interfollicular compartments, which are relatively independent although exchanges may occur between them [44, 50, 107]. Guttate hypomelanosis and repigmenting vitiligo are readily visible examples of the perifollicular compartment [18].

Hair, even of normal density, does not fully protect skin against UV. The reduction in hair fullness is accompanied by a dramatic failure in the hair shielding effect on the interfollicular epidermis. This is probably the first preliminary step of scalp actinodermatosis before increasing both the Sun-induced dermal remodeling and the neoplastic risk. It remains a major challenge to decipher which epidermal melanization pattern results from alopecia and which one may cause alopecia through the SIPS mechanism. In any case, it was framed as a hypothesis that, similar to the epidermis [7], the speckled perifollicular melanoderma may act as a photoprotection for the follicular stem cells (FSCs). FSCs have a high proliferative potential but very low levels of ongoing cell cycling [34, 100]. They have multipotency and high regenerative potential. They have been localized in the isthmus-bulge portion of the hair follicle [34, 100].

12.6 Teloptosis, Hair Eclipse Phenomenon, and Actinic Telogen Effluvium

Exposure to UV of solar origin determines a variety of transient and cumulative skin responses. Chronic effects of UV light can be observed histologically both

in the epidermis and dermis of the scalp. One of the most obvious and undisputable hallmarks of these events is solar elastosis [59]. In these respects, quantifying the deposition of lysozyme onto abnormal elastic fibers is a good means to assess the effects of chronic UV exposure [1, 31–33, 40, 46, 59, 79]. In contrast with many recognized epidermal and dermal UV damages, the UV impact on human pilosebaceous follicles has seldom been explored [11, 53, 54, 59, 91, 96, 110]. However, seasonal moulting influenced by light exposure cannot be denied in the animal [25]. Furthermore, cyclic hair shedding in humans may also be influenced by a perennial chronobiological rhythm apparently regulated by the intensity of UV exposure [53]. Indeed, transient telogen effluvium may be a delayed summertime consequence [11, 53].

Following a putative UV-induction of the catagen phase [12, 20, 36, 87], two basic mechanisms might increase telogen shedding. Any synchronization of the hair cycle by shortening the anagen phase duration might be operative. It is also possible that the duration of the telogen phase is controlled by the induction of teloptosis (also called exogen phase) [48, 54, 90, 91]. In these instances, synchronized hair shedding is not always a step of progressive baldness [10]. In fact, it is debatable to what extent telogen effluvium, whether sporadic or chronic, represents a pathway into permanent alopecia or represents a self-limiting moult without any consequence or long-term hair density [10, 51, 53]. However, those affected individuals express, not infrequently, anxiety that alopecia will prevail sooner or later [47, 104]. In addition to uncertainties regarding the UV impact on the normal hair cycling and fullness, some accounts are also uninformative about the effect of UV on an already ongoing chronic effluvium. The currently recognized three main representatives of these disorders are androgenetic alopecia, diffuse telogen effluvium in women, and senescent baldness [51, 54].

12.7 Photosensitive Scalp Disorders

UV exposure exerts some immunomodulatory effects in the skin. There is a balanced depletion in Langerhans cells and an income in Factor XIIIa-positive dendrocytes migrating into the epidermis [55].

A number of photopathological conditions occur on the scalp. Baldies may suffer from photosensitive disorders of the scalp including dermatomyositis, lupus erythematosus, and a few distinctive UV-sensitive disorders including the so-called red scalp syndrome and the erosive pustular dermatosis of the scalp. Dermatomyositis is often pruritic and associated with diffuse alopecia [29]. In this condition, deposits of acid proteoglycans are present throughout the dermis. Lupus erythematosus typically presents as focal alopecia on atrophic macules studded with follicular plugging, telangiectasia, and mottled melanoderma. The end result is scarring alopecia. A squamous cell carcinoma eventually develops on these sites [92]. The red scalp syndrome refers to a peculiar condition that is often itchy and burning [94, 96]. Sun exposure exacerbates this condition, which is notoriously refractory to antiinflammatory treatments. A relationship was suggested with the female pattern of androgenetic alopecia and with an early stage of scarring alopecia. Erosive pustular dermatosis of the scalp is a distinctive clinical entity of the elderly [6, 69, 96, 99]. Extensive pustulation leads to erosions and progressive scarring alopecia. Scalp injury often initiates the dermatosis on the bald scalp particularly exposed to UV light [16]. It is a corticosteroid-responsive and an immune downregulator-responsive disorder.

Propionibacterium spp. collected in the hair infundibulum produce porphyrins [28], which under UV light photoactivation lead to ROS. Nitric oxide and pro-inflammatory cytokines are also released [76]. In addition, keratinocytes are altered by UV-induced SIPS and participate in the same biological process. As a result, perifollicular microinflammation develops probably leading to dandruff [58, 62] and hair effluvium [27, 38, 57, 61]. *Malassezia* spp may participate in the process as well as the antimicrobial peptides of innate immunity [67]. Antimicrobial treatments are quite likely to improve this condition [38, 66].

12.8 Androgenetic Alopecia and Stress-Induced Premature Senescence of the Scalp

Androgenetic alopecia is an androgen-driven condition that represents a physiological response of the hair follicle rather than a disease. The progressive alopecia

decreases the hair protection of the scalp to environmental UV light. Therefore, it is not surprising that the signs of photoaging are found in bald-headed subjects. In addition, it has been suggested that the UV exposure could aggravate the hair loss in androgenetic alopecia, thus inducing a vicious circle [59, 96].

12.9 Trichodynia

Trichodynia is a scalp discomfort possibly influenced by UV light [61]. It is a quite frequent condition perhaps related to the release of the neuropeptide substance P by a perifollicular microinflammation [98]. A correlation was suggested with alopecia and presence of scalp telangiectasia [105].

12.10 Actinic Field Cancerogenesis

Exhibiting suntan has long been synonymous with beauty, healthy condition, and dynamism in Westernized culture. This gave rise to the development and uncontrolled use of high-pressure UVA sources. Moreover, exposures to solar radiation during holidays have increased over the years, encouraged by the use of UVB sunblockers showing little protection against UVA. Unfortunately, in addition to cutaneous photodamage, UVA cause more serious cellular lesions in the long term and do so in a much more insidious way than UVB.

Various chromophores are capable of absorbing UV energy and generating ROS. These compounds cause damage to membrane lipids and proteins, and lead to DNA destruction. Cell mutation is the final stage of the deleterious UV effects. UVA doses as small as 1–2 MED and repeat suberythemal UVA doses can lead to DNA damage, photodyskeratotic cells, p53 mutation, epidermal ferritin expression, and tenascin expression in the dermal extracellular matrix [2, 79].

The concept of field cancerization was born half a century ago [80]. It refers to the fact that environmentally induced cancer is usually rooted in an area presenting biological changes already linked to the process of neoplasia [4, 9]. As UV are mostly involved in the development of actinic keratoses and squamous cell carcinomas on the scalp [37, 72, 73], the term actinic field carcinogenesis seems better suited to describe this condition. However, other neoplasms can occur. In particular, the scalp is a body site where malignant melanoma develops, even on nonbalding areas [14, 71]. The role of UV could be due in part to the generation of procarcinogen photoproducts from eumelanin and particularly from pheomelanin.

Diverse biological mechanisms underlie UV-induced skin cancers. They participate in a multistep process involving DNA alterations. Any mutation in specific key genes potentially contributes to cancerogenesis. In particular, genes controlling cell growth in a direct way or through immunosurveillance mechanisms are important for the survival, growth, and progression of photo-induced malignancies. In addition, telomerase activity helps cells to survive for a long time. Many skin neoplasms including those resulting from photocarcinogenesis show a high telomerase activity [101].

Actinic field carcinogenesis is a concept supported by some biological features. Before neoplasia are fully developed over the concerned area, for instance the scalp, more subtle suggestive changes may appear at the clinical inspection under UV illumination [52, 70, 73, 108]. The ULEV method reveals an unusual melanization pattern combined with a peculiar discrete scaly pattern corresponding to annular and circinate rims [73]. They surround small macules with low melanin density.

Vitamin D3 derivatives and imiquimod can be used to control photodamage [96] and different steps of actinic field carcinogenesis [63, 86, 99, 102, 103].

12.11 Hair Shaft Photodamage

The hair shaft is altered following UV exposure [5, 15, 45, 93]. Hair lightening by Sun exposure during summer is particularly noticeable on light-colored hair and on hair exposed to Sun and sea-spray. Such effect of sunlight also leads to fragilization of the hair shaft, particularly following some chemical aggression [21–24, 26, 93, 106].

Hair keratin at the difference with melanin does not absorb visible light in a wide range of wavelengths. Consequently, when hair is subjected to

intense radiation with visible light, the energy absorbed by melanin is in part transformed into heat and may destroy chemical bonds. This second type of effect only occurs when the received energy is greater than that of the chemical bonds. The energy of light is given by Planck's law: $E = hv = hc/\lambda$, where E is the energy, h the Planck constant, v the frequency, c the speed of light, and λ the wavelength. With a visible wavelength at 532 nm, the energy supplied exceeds 50 kcal/mol, which is greater than the bonding energy of melanin (ca. 40 kcal/mol). Therefore, lightening hair with visible light without damaging the hair shaft could be obtained under three conditions [109]. The light emission should be strictly limited to the visible spectrum as keratin also absorbs in the infrared and UV ranges. There should be sufficiently high energy to destroy the chemical bonds of melanin. The shortest possible duration of emission should be applied so as to avoid its conversion into heat (in fact 1 μs; at most, the thermal relaxation time of melanin). This working hypothesis suggests the use of pulsed light sources, delivering a large amount of energy over a very short space of time.

Hair resistance to environmental light can be tested using a sunlight simulator delivering radiation with a spectral distribution close to that of sunlight in its visible and UV ranges [109]. Using special filters, the combination of radiation power, exposure time, and intensity of UV radiation should be controlled. In addition, the assessment of light/rain effect can be tested combining exposure to Sun simulator light and repeated water spraying [109].

12.12 Conclusion

There is mounting evidence that the scalp, hair shafts, and hair growth are affected by sunlight and any source of UV. Multiple biological mechanisms appear to be operative. The diversity of the induced changes ranges from aesthetic concerns to hair loss and carcinogenesis as well. Both the genetic susceptibility and the individual behavior regarding Sun exposure have a profound influence on the clinical consequences.

Take Home Pearls

- Hair does not fully protect from UV effects.
- Both UVA and UVB in combination alter the scalp.
- UV exposures probably influence scalp chronobiology.
- Light-modulated hormones (e.g., melatonin) and locally released mediators combine their effects on hair biology.
- The different types of subclinical melanoderma are early indicators of the UV effects on the scalp.
- UV exposures can modify some aspects of the hair cycle increasing the severity of specific hair loss disorders.
- Repeated UV exposures of the scalp progressively lead to a condition called actinic field carcinogenesis. With aging, skin cancers may develop.
- The texture and color of hair are affected by UV light combined or not with sea-spray.

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Hair Photoaging

13

Won-Soo Lee

Core Messages

- Hair photoaging is shown to consist of a number of concurrent processes that result in chemical and physical changes in fiber properties. Lipid oxidation, disulfide bond cleavage, tryptophan degradation, and cysteic acid formation lead to an increase in fiber porosity, loss of mechanical strength, and an increase in surface roughness. Hair exposed to sunlight is claimed to be more brittle, stiffer, and drier than before irradiation and exhibits a reduced water absorption capacity.
- Photochemical alteration includes the breakdown of disulfide bridges within the structural units of hair and the establishment of new intra and intermolecular cross links via reaction of carbonyl groups with protein amino groups within and between structural units, thereby decreasing structural integrity. These reactions most likely lead to a gradual increase in brittleness and a gradual loss of structural differentiation. Photodegradation of cystine occurs through the C–S fission pathway and the highest level of photodegradation occurs in the cuticular region, where cystine is present at its highest concentration.
- For hair damaged by sunlight, in most cases, the amino acids of the cuticle are altered to a greater extent than the amino acids of the cortex, because the outer layers of the fiber receive higher intensities of radiation. Proteins of the cuticle are degraded by ultraviolet A and ultraviolet B, but much less by visible (VIS) light.
- Hair pigments function to provide some photochemical protection to hair proteins. Hair pigments accomplish this protection by absorbing and filtering the impinging radiation and subsequently dissipating this energy as heat. However, in the process of protecting the hair proteins from light, the pigments are degraded or bleached.
- Dark hair is more resistant to photodegradation than light hair, because of the higher photostability of eumelanin when compared with pheomelanin. But, hair damages caused by ultraviolet (UV) exposure are related not only to the melanin type of each hair but also to the total amount of melanin. Pheomelanin is far more sensitive to UV light than eumelanin, though these two types of melanin are similarly sensitive to VIS light.
- UVA irradiation can penetrate deeply into the cortex, so photochemical changes, including cuticles and cortex together, may appear greater after UVA irradiation. On the other hand, UVB causes severe morphological damages, especially confined to the hair cuticles because of its restricted depth of penetration.
- Integral lipids (ILs) of hair fibers are degraded by UV light as well as by VIS light, helping to explain the weakening of the cell membrane complex (CMC) exposed to light radiation.

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13.1 Introduction

Although being quite less important than the study of skin photo damage, from the point of view of health risks, healthy hairs are associated not only with beauty but with overall self-esteem [53]. A physicochemical aging of the fiber resulting from external stimuli is imposed on the hair during its life span of, on average, 3–4 years. The images obtained by scanning electron microscope showed that the regular surface of the cuticle close to the root becomes highly eroded toward the hair end. Another technique showed that the degree of oxidation of a hair (using the cysteic acid level as indicator) progressively increased from the root to the hair end [9]. Hair damage is a generally recognized term that encompasses a variety of attributes, among which are dryness, ease to breakage, split ends, a coarse feel, lack of manageability, and lack of luster [75]. Hair aging comprises degradation of the hair shaft, which involves progressive degeneration of the hair fiber from the root to the tip. Human hair is constantly subjected to repeated environmental assaults, commonly termed "weathering," which is aggravated by various extrinsic factors. Extrinsic factors that cause weathering include sunlight, water, dust, friction, hair combing, and cosmetic hair treatment such as hair dyeing or permanent waving. These factors cause extrinsic hair shaft aging in addition to natural intrinsic hair shaft aging [42, 73].

Among the natural aggressors, sunlight in particular, UV rays play an important role in hair aging. This is what we call photoaging, which is the subject of this chapter. In light-induced aging, the hair becomes paler in color and changes occur in the surface condition, which impair the softness and shine of the hair. Hence, light modifies the cosmetic properties of hair. Hair fibers therefore need to be protected from the light [9]. UV light is the most energetic solar radiation reaching the Earth's surface. It is the precondition of life on Earth, but may also cause disease and destruction [5]. Exposure to light radiation is known to cause many undesirable effects. Dyes, fragrances, and carbomer type thickeners exhibit varying degrees of instability upon exposure to light [75]. Although it is not immediately perceived, UV damage to hair fibers plays an integral role in the overall aspects of hair damage [75]. Hair exposed to natural sunlight showed a decrease in tensile strength and was more prone to alkaline attack (alkaline solubility). VIS light increased the alkaline solubility and more than doubled the cysteic acid content of the hair when compared with unirradiated hair [16].

Sunlight-induced hair damage is difficult to avoid during daily life. Concerns about the effects of sunlight on hair are emerging recently. Photochemical degradation of hair results in the attack on both hair protein and hair pigment. The most obvious effect of sunlight is that of decoloration. Changes in hair fibers induced by UV light are largely composed of physical and chemical changes. As for physical changes, dryness, reduced strength, rough surface texture, loss of color, decreased luster, stiffness, and brittleness may occur. With respect to chemical changes, hair proteins, lipids, and hair pigments can change [34]. Lipid oxidation, disulfide bond cleavage, tryptophan degradation, and cysteic acid formation lead to an increase in fiber porosity, loss of mechanical strength, and an increase in surface roughness [18, 33,62]. Photoyellowing or photobleaching is induced by UV irradiation with shorter (~300 nm) or longer (~398 nm) wavelengths [45]. The photochemical effects on hair color are strongly dependent on the presence of melanins and chromophores in the hair [27]. Pheomelanin is more sensitive than eumelanin [29]. Humidity may increase the effects of UV light [45]. Hair pigments function to provide some photochemical protection to hair proteins, especially at lower wavelengths. Hair pigments accomplish this protection by absorbing and filtering the impinging radiation and subsequently dissipating this energy as heat. However, in the process of protecting the hair proteins from light, the pigments are degraded or bleached [29]. UV light and oxygen affect not only the melanins but also the amino acids and fatty acids in the hair and on the cuticle. Amino acids can be destroyed or polymerized. The aromatic amino acids are more sensitive than other amino acids [45].

13.2 Photochemical Changes of Hair

13.2.1 Protein Damage

Among the keratin amino acids, tryptophan, cystine, tyrosine, and histidine are more susceptible to photodegradation. The total amount of these amino acids depends on hair type. Male hairs have more cystine than female and, usually, dark hairs have more cystine

than light hairs [59]. According to Bertazzo et al. [6], the amount of tryptophan in dark brown and black hair is greater than in blond hair. The highest tryptophan concentration is found in gray and white hair, indicating that tryptophan concentration in hair increases with age. Pande and Jachowicz [54] used fluorescence spectroscopy to monitor the decomposition of tryptophan in hair. They demonstrated that photodegradation to tryptophan does occur in hair, and they speculated that tryptophan photodamage may lead to or make other amino acids more sensitive to photodegradation. Tryptophan is an integral part of keratin and it absorbs UV rays at a maximum wavelength of 280 nm. While no great difference was found between wet or dry fibers exposed to UV radiation, a big difference was found between water-soaked fibers and fibers soaked in mineral oil. The rate of destruction of tryptophan was found to be much lower in nonpolar media (mineral oil) than in water [23].

For hair damaged by sunlight, in most cases, the amino acids of the cuticle are altered to a greater extent than the amino acids of the cortex because the outer layers of the fiber receive higher intensities of radiation [59].

Light radiation has been shown by Beyak et al. [7] to decrease the wet tensile properties of human hair. They relate these effects to the total radiation that the hair is exposed to rather than to any specific wavelength. However, hair proteins have been shown by Arnaud et al. [4] to absorb light primarily between 254 and 350 nm. Several amino acids of hair absorb light in this region, and these amino acids are the most subject to degradation by light. The following amino acids have been shown to be degraded by light radiation on wool fiber [30, 40]: cystine and methionine (the sulfur-containing amino acids); the aromatic and ring amino acids phenylalanine, tryptophan (often associated with photoyellowing of wool), histidine, and proline; and the aliphatic amino acid leucine. Hoting et al. [29] showed that the proteins of the cuticle are degraded by UVB and UVA, but much less by VIS light, and that cystine, proline, and valine are degraded more in light brown hair than in black hair. In other words, the photoprotective effect is much better in dark hair than in light hair. Oxidation at the peptide backbone carbon has been shown to occur from UV exposure both in wool [24] and in hair [58], producing carbonyl groups (alpha keto acid/amide) and amide groups. The formation of carbonyl groups is favored in the dry state reaction more than in the wet state. This reaction is similar to the oxidative damage to proteins and mitochondrial decay associated with aging [13].

In a recent study, Inoue et al. [32] have suggested that ubiquitin could be an indicator of hair damage. Stable protein portions in normal hair are transformed to labile protein, the internally formed soluble protein, which is accumulated in damaged hair shaft. The major components of the labile protein are ubiquitins. Labile protein must be associated with hair damage and could possibly provide marker for hair photodamage [34]. S100A3, a unique protein among all members of the calcium-binding S100 family, is specifically expressed at the inner endocuticle of human hair fibers. Upon hair damage, S100A3 is released from hair fibers and possibly destabilizes the hair tissue architecture [31]. S100 proteins represent the largest subgroup in the EF hand Ca2þ binding protein family [20]. Among the 20 members of the S100 protein family, S100A3 is exceptional with regard to its high cystine content (10 out of 101amino acids) [17]. S100A3 protein is specifically expressed in the cuticular cells of human hair shaft [30] and in the cuticle of murine pelage follicle [38]. In human hair fiber, the protein is segregated in the endocuticle of cuticular cells and in the matrix that surrounds macrofibril bundles in cortical cells [69]. Therefore, it was postulated that in the matured hair shaft, S100A3 is possibly cross-linked to hair keratin by disulfide bridges. Thus, S100A3 could possibly provide structural integrity to the hair fiber and must be associated with hair damage [31]. S100A3 is released from the cuticle of hair fibers during washing and rinsing, especially from chemically treated or UVB irradiated fiber [31].

13.2.2 Pigment Change

According to Borges et al. [8], black hair contains more or less 99% eumelanin and 1% pheomelanin, both dark brown and blond hairs contain 95 and 5%, and red hair 67 and 33%, respectively. Generalizing, dark hairs have more melanin and more photosensitive amino acids than light hairs. Melanin can attribute photoprotection to hair protein, but only in the cortex. As dark hair has more photosensible proteins than light hair, they can show a greater protein loss than light hair in the cuticle region. In the cortex, even though dark

hair has more photosensible proteins than light hair, they also have more melanin to absorb the UV radiation [52]. The nonpigmented hairs are also sensitive to light radiation and if exposed to UV radiation for a sufficient period, they will show lower levels of cystine and correspondingly higher levels of cysteic acid, particularly in their outer layers [23].

Red hair was found to lighten to a similar extent by irradiation from both UV and VIS light. On the other hand, under the same irradiating conditions, blond hair was lightened by VIS light but did not lighten by UV light until it was washed after irradiation [67]. These different photolightening behaviors of red and blond hair are supposed to be due to the differences in their melanin compositions. The dominant type of melanin in red hair is pheomelanin, whereas blond hair contains both eumelanin and pheomelanin, mainly with eumelanin. Vincenci et al. [74] studied red hair, observing that the amounts of pheomelanin and eumelanin vary with sex, age, and color shade. It has been proven that chemically intact melanin in red hair is considerably more photolabile to UV light than VIS light. Also, it is much more easily decomposed by UV light than melanin granules in blond hair, although they are both similarly decomposed by VIS light. This indicates that pheomelanin is far more sensitive to UV light than eumelanin, though these two types of melanin are similarly sensitive to VIS light [68].

13.2.3 Photoaging According to Different Light Spectra

Human hair can be considered as a two-component system regarding its interaction with VIS and UV radiations. The keratins are transparent in the VIS region of the light spectrum, but a few amino acids, tryptophan, cystine, tyrosine, and histidine, interact with UV. Melanins, which are located only in the cortex, interact with both VIS and UV radiations [53]. There are conflicting conclusions on the photochemically active region of the sunlight, e.g., with reference to the brightening of the color of human hair. Reese and Maak [57] attribute the bleaching action of the sunlight exclusively to the VIS range of the sun's spectrum. In contrast, Tatsuda et al. [71] demonstrated a bleaching effect of UV radiation on black hair. According to the extensive study conducted by Hoting et al. [28], sunlight-induced color changes in hair are more extensive in light brown than in black hair. The most obvious photobleaching effect occurs within the VIS range of sunlight. Furthermore, UVA and UVB also cause a lightening in light brown hair. The proteins of the cuticle are especially impaired by UVB and UVA, but only slightly by VIS light. The composition of the amino acids in the cuticle is more strongly altered than in the cortex since greater intensities of radiation can act in the outer cell layers. The changes in the cuticle proteins do not show any correlation with the hair color as cuticle layers are devoid of pigments. In contrast, differences between the irradiated proteins of light brown and black hair cortex (melanin-rich areas) are readily detectable. In particular, the cystine, proline, and valine residues in whole light brown hair are more markedly degraded by UVA and UVB than in whole black hair [28]. Infrared (IR) spectroscopic investigations (FTIR) support the conclusions drawn from the amino acid analyses with respect to the radiation-induced formation of cysteic acid in the cuticle. The highest contents are reached by irradiation with VIS light and UVA to the same extent in light brown as in black hair cuticle layers [28].

According to the study conducted by Nogueira and Joekes [52], UVB radiation is the principal radiation responsible for hair protein loss and that UVA radiation is the one responsible for color changes, disregarding the hair type. Color changes depend on hair type, being more pronounced for light colored hairs. Although lightening every hair type is the main effect of radiation, significant variations in all color parameters are observed after both sun and artificial lamp irradiation. When UVB radiation hits hair, it has to penetrate a layer of absorbing molecules of about 5 μm thickness, the intensity decreasing exponentially [66]. Since the absorption of UVA radiation by these molecules is about five times smaller, it can be considered that UVA radiation, as well as VIS radiation, are totally transmitted in the cuticle region [52]. Considering that intact hair cuticles are of 6–10 layers, each with a thickness of 0.3–0.5 μm, UVB seems to affect mainly the cuticles. This suggests that hair damage by UVB irradiation is mainly confined to the superficial layers of the hair shaft, the cuticle layers. Jeon et al. [34] related this to the penetration depth of UV light mentioned earlier. These authors described that UVA irradiation can penetrate deeply into the cortex, and so photochemical changes, including cuticles and cortex

together, may appear greater after UVA irradiation. On the other hand, UVB causes severe morphological damage, confined to the hair cuticles because of its restricted depth of penetration [34]. In their study, morphological evaluation showed relatively more destructive cuticular changes after UVB irradiation than after UVA, while disruptions of the intercellular lipid layer (LL) showed similar results between UVA and UVB irradiation. However, in labile protein analysis [32], damaged labile hair proteins were much more observed after UVA irradiation than after UVB irradiation [34].

UV light has been generally considered to be the cause of hair damage, but recent studies have revealed that VIS light also contributes to hair damage [27–29]. Although the energy of a photon of UV light is higher than that of VIS or IR light, the number of photons of UV in sunlight is much lower than for VIS or IR light. The ratio of UV (280–400 nm): VIS (370–780 nm): IR (750–2,800 nm) intensity is roughly 1:10:10. When the total radiation is considered, the relative proportions of UV, VIS, and IR are 4–6%, up to 52%, and up to 42%, respectively [75]. The shorter the wavelength, the greater the energy of the radiation; even small portions of very energetic radiation can have dramatic effects [19]. Hoting et al. [27–29], studied the effect of these light sources using their own irradiation equipment with the spectral distribution corresponding to natural sunlight. They reported that VIS light primarily caused decomposition of melanin and lipids while UV light mainly caused decompression of proteins.

13.3 Pathomechanism of Photoaging

Human hair is composed of keratin, a group of insoluble cystine-containing helicoidal protein complexes, which form 65–95% of hair composition by weight. The greatest mass of the hair shaft is the cortex, which is responsible for the mechanical properties of the fiber [60]. These properties are dependent on time, temperature, and humidity [83]. Surrounding the cortex is the cuticle, a layer of overlapping, keratinized scales, which can account for 10% of hair fiber by weight and has the role of protecting the fiber against environmental and chemical damage [14]. As the cuticle protects the cortex, damage in this region generally occurs after extensive damage to the hair cuticle. Bleaching and sun exposure are known to cause damage to both hair regions [63].

Black hair is more photostable than blond hair. Black hair seems well protected against UV light, and light brown hair is obviously damaged by a wide range of the natural sunlight. The protective action of melanin granules is limited to the melanin-rich cortex of black hair, which shows only a slight modification of fiber proteins under irradiation; the melanin-poor cuticle proteins of black as well as of light brown hair are modified to a similar extent. The photodegradation of cystine is the most conspicuous alteration on the amino acid residue of the whole fiber, to which additional consequences for the hair are attributable: loss of fiber strength and water infiltration that subsequently creates favorable conditions for further photooxidative reactions by the dissolved oxygen [78]. Robbins and Bahl [58] have examined the effects of sunlight and UV radiation on disulfide bond in hair via electron spectroscopy for chemical analysis [58]. Both UVA and UVB radiation were shown to oxidize sulfur in hair. The primary oxidation occurs closer to the hair fiber surface, producing a steep gradient of oxidized to less oxidized hair from the outer circumference of the hair to the fiber core.

Light radiation causes degradation of cystine, but the exact mechanism is not well known. Literature suggests that the photodegradation of cystine occurs through the C–S fission pathway and is different from the chemical oxidation of cystine that proceeds mainly via the S–S fission route [59]. Savige and Maclaren [64] also suggest the C–S fission route as the preferred route for photochemical degradation of cystine and for other pure disulfides. In general, for the photochemical reaction, if the pH is neutral or alkaline, then the C–-S fission route is the preferred one; however, if the pH is acidic, then the hemolytic or S–S fission route is more likely to occur. Changes on mechanical properties of irradiated human hair correlate with the photodamage of the hair proteins [57, 71]. It was shown that the C–S bonds of cystine are cleaved upon UV radiation [71]. The decrease in the cystine content, however, does not necessarily imply an increased swelling of the hair, since cross-linking of amino acid residues may occur as well [78]. With long-term irradiation, there is a progressive decrease in the total recovery of amino acids after hydrolysis, except for alanine, glycine, and arginine. However, there is no clear pattern of protein degradation [78]. For the S–S fission route, the main end

product is sulfonic acid. For the C–S fission route, the main products are the S sulfonic and sulfonic acids [64]. However, ultimately, S sulfonic acid is degraded by light to sulfonic acid [64]. These results suggest that the mechanism for the radiation-induced degradation of cystine occurs through the C–S fission pathway and is different from the chemical oxidation of cystine that proceeds mainly via the S–S fission route. Differentiating the effect of UVA and UVB radiation on hair structure, Ruetsch et al. [62] also studied hair photodamage under conditions of prolonged exposure to UV radiation, and hair has been found to undergo substantial changes, both chemical and morphological. These changes were more pronounced, as more humidity/moisture was available during exposure. The number of hours of exposure used in this study was up to 700 h. Photooxidation occurs at the cysteine C–S bond to yield one mole of cysteic acid among the products of reaction, and the mechanism is thought to be free radical in nature [49]. This mechanism is different from chemical oxidation that follows the S–S scission pathway yielding two moles of cysteic acid. Photodegradation of hair proteins was more pronounced in unpigmented/blonde hair, the highest level of photodegradation occurring in the cuticular region, where cystine is present at its highest concentration. Prolonged UV irradiation produced the thinning and the fusion of cuticular cells; the proposed theory is that the outer proteic layers would photodegrade to smaller, lower molecular weight peptides, which would then diffuse into the hair structure when enough humidity would allow for the fiber's swelling [49].

The photochemical degradation by VIS light of eumelanin, the predominant pigment of black and light brown hair, is relatively small and can be shown quantitatively (gravimetry) and qualitatively (color measurements, IR spectroscopy) [29]. On the other hand, a mixture of pheomelanin and eumelanin, the pigment of light brown hair, appears to be affected by all segments of sunlight, particularly by UVA and VIS light. The irradiation brings about drastic degradation of the granules and substantial changes in the melanin polymer [29]. IR investigation of the melanin of irradiated light brown hair suggests extensive destruction of the quinine system, which according to Crippa [12] is essential for the photoprotective effect of the melanin. In the case of eumelanin, the dihydroxyindole moieties are prone to irradiation [29]. The higher photostability of the eumelanin, particularly the limited degradation of the quinine structure, suggests that eumelanin has a better photoprotective effect on hair than pheomelanin [29]. Eumelanin ring opening result from free radical reaction. Slawinska and Slawinski [65] have suggested that photochemical degradation of melanin occurs through similar peroxide intermediate. The first step in the photochemical degradation of the eumelanin chromophore probably involves excitation to a radical anion and then attack by the oxygen radical anion on the 0 quinone grouping. Ring opening of the six-membered ring indolequinone species then follows. A related scheme may be involved for the photochemical degradation of pheomelanins. However, Arakindakshan et al. [3] have shown that pheomelanins are more easily induced to an excited state than the brown black eumelanins. Hoting et al. [29] have shown that the pigment of light brown hair is affected by UVA, UVB, and VIS light, but eumelanins are much light-tolerant and provide a greater photoprotective effect.

UV radiation induces the formation of oxy radicals such as superoxide and hydroxyl. These species have one unpaired electron in an outer orbital giving them a very powerful aptitude to react, especially with molecules having a double bond in their structure, such as unsaturated lipids [1]. Chemically, these changes are thought to be caused by UV light induced oxidation of the sulfur-containing molecules within the hair shaft. Melanin has an intrinsic electron spin resonance (ESR) signal that increases significantly when irradiated with UV and VIS light. In the presence of oxygen, superoxide is produced, which dismutates to hydrogen peroxide. This leads to the formation of hydroxyl radicals in the presence of trace amounts of metal ions. Oxidation of the amide carbon of polypeptide chains also occurs, producing carbonyl groups [14]. This process has been studied extensively in wool, where it is known as photoyellowing [35, 48].

Although there is a general agreement in the literature that melanin gives photochemical protection to hair, many contradictions are found about the photostability of pheomelanins and eumelanins. Hoting et al. [28] state that dark hair is more resistant to photodegradation than light hair, because of the higher photostability of eumelanin when compared with pheomelanin. On the other hand, Wolfram and Albrecht [79] claim that pheomelanin is less vulnerable to photodegradation than eumelanin. But, hair damages caused by UV exposure are not related only to the melanin type of each hair. Most likely,

the smallest protein loss observed for black hair is associated with the greater amount of melanin in this hair when compared with other types of hair. In other words, a hair with more pigment granules will show a smaller protein loss when exposed to the UV range that is absorbed by both hair pigments and hair proteins. Also, protein loss is observed after only 56 h of UVB radiation exposure, when probably only cuticle proteins are damaged, and this is also a clue that the photoprotective effect of melanin is related to the total amount of melanin in the hair and not just with the melanin type [52]. Contradictions are also found in the literature about the effect of specific wavelength ranges of UV radiation on hair properties. Authors generally attribute hair damage to the total UV range of the solar spectrum [59]. Pande and Jachowicz [54] indicate that UVA and VIS radiation do not cause direct damage to hair because they are not absorbed by hair proteins. Ratnapandian et al. [56] studied the effect of humidity on the mechanical properties of hair exposed to UV radiation. The authors observed that greater damage happens when hair is exposed to very high or very low relative humidity and that the mechanical properties of hair are less affected when hair is exposed to 30% RH.

13.4 Main Features of Photoaging

Regarding the evaluation of photoaging, another note of considerable importance is the lack of correlation between the radiation emitted by the sun and that emitted by the solar simulator. It is known that even small differences, at very high energy frequencies, can result in big differences in results when comparing the effect of natural light exposure to that of artificial light [75]. There is no standard protocol on how to expose human hair to light and/or UV radiation; there is no standard test for assessing the damage that UV radiation causes [75]. The first evidence of natural aging includes changes in the visual properties of hair, loss of color and shine, and in its surface properties, wettability and softness [37]. Images obtained by transmission electron microscopy of hairs both exposed and unexposed to sunlight show that melanin pigments have been loosened from their envelope and that some have vanished. This explains the loss of color. The microfibrillar structure appears unaltered.

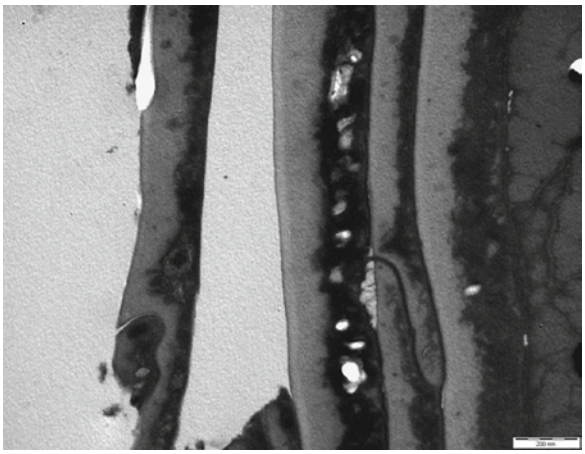

Fig. 13.1 Conventional transmission electron micrograph of hair fiber illustrating extensive damages of endocuticle of cuticle cells and resultant detachment of several cuticle layers with ultraviolet (UV) A irradiation

However, cuticle cells are missing or detached, the endocuticle is affected, and intercellular adhesion seems to be impaired (Fig. 13.1). Morphological changes undergone by light-exposed cuticle explain the rise in the friction coefficient of a hair, the increased fragility of the cuticle, and increased porosity [10]. The hair has thus become more vulnerable to mechanical or chemical treatments. Degradation of the cortex was shown by a loss in mechanical resistance of light-exposed hair [7]. Hair exposed to sunlight is claimed to be more brittle, stiffer, and drier than before irradiation, and exhibits a reduced water-absorption capacity [25, 72]. Both the tensile strength and the elongation at break are diminished in irradiated hair [7].

Long-term UV exposure causes severe chemical degradation to the hair proteins. As previously indicated, the damage is so extensive that structural differentiation is diminished. This physicochemical degradation usually occurs at a higher level in the hair fiber periphery with a gradient to a lower level of oxidative damage deeper inside the fiber. Such damage leads to unusual fracture patterns during extension. This includes the breakdown of disulfide bridges within structural units and the establishment of new intra and intermolecular cross-links via reaction of carbonyl groups with protein amino groups within and between structural units, thereby decreasing structural integrity. These reactions most likely lead to a gradual increase in brittleness and a gradual loss of structural

differentiation [59]. Subsequent exposure to aqueous alkaline solution or to peroxide solution leads to rapid dissolution of the affected areas even during short-term exposure to peroxide solutions (bleaching solutions). Longer exposure of these UV-radiated fibers with oxidizing solution leads to dissolution/elimination of scale differentiation and dissolution of the melanin granules [59]. Robbins and Kelly [61] have analyzed amino acids of both proximal and distal ends of human hair and have shown significantly more cysteic acid in tip ends. They attribute this change to weathering actions, specifically to UV radiation. This same study also found significant changes in tyrosine and histidine similar to the weathering effects in wool fiber. Decrease was also reported in the lysine content in this study on hair weathering.

A well-known and most obvious effect of the photoaging of human hair is hair lightening, an effect that is accelerated by moisture [71, 72, 79]. The extent of this photochemically induced color change is dependent on the nature of the hair pigment and is understood to involve an oxidative attack on the eumelanin or pheomelanin melanosomes [12]. The most perceptible is the change in fiber coloration as a result of photoreactions of the natural pigments, either eumelanin or pheomelanin [27, 79], as well as photodecomposition of artificial hair dyes [11, 27, 46]. As far as the latter is concerned, we refer to permanent oxidative hair color, which is expected to last indefinitely and resist washing and weathering. The time scales of photoreactions require exposure times on the order of at least 200 h to produce a perceptible shift in color shade, especially for highly pigmented fibers. In addition, natural pigmentation is resistant to washings and artificial oxidative hair color fades at a much faster rate, with dark auburn shades typically undergoing perceptible lightening within 8–10 h of irradiation and one shampoo application [44]. It is also worth mentioning that it has been found that artificial coloration has a photoprotective effect on hair keratin [55]. Even though hair dyes are damaging to the hair shaft, the photoprotective effects of replacing hair shaft pigments may offset some of this damage [15]. It is generally agreed that artificial hair color instability, caused by weathering and habitual grooming practices, has several underlying components, such as (a) removal of a dye from hair during shampooing, (b) photodecomposition of hair color chromophores as a result of irradiation, and (c) thermal decomposition of the dye. In addition to this, the hair coloring dyes exhibit different sensitivities to the UVB, UVA, VIS, and IR portions of solar radiation. It has been demonstrated in the literature that VIS and UVA light are mostly responsible for the photofading of artificial hair color [27].

13.5 Photodamage to Integral Hair Lipid

Hair lipid consists of surface or external lipid and internal or IL. In addition, part of the IL is free lipid, and part is structural lipid of the CMC. The CMC is laminar in structure and is composed of both protein and lipid layers; however, this structural lipid is not phospholipid like those normally associated with bilayers of cell membranes [50]. Using the example of cholesterol and free fatty acids (FFAs), it could be demonstrated that the VIS range of sunlight destroys IL to a considerably higher extent than exposure to UVB and UVA ranges. The photochemical destruction of lipids is largely retarded by the pigment eumelanin [26]. Cuticle cells are covered with a thin lipid layer (LL) covalently bonded to hair proteins [2]. The chemical composition of IL is different from those of epidermal or sebaceous lipids [41]. The lipid content of human hair comprises 0.7–1.3% of the total components of hair. The LL in hair is the only continuous structure that plays a key role in the maintenance of hair integrity including hydrophobicity and stiffness [36]. Cuticle cells in hair are abundant in fatty acids unlike the keratinized area of epidermis or sebaceous gland, and about 40% of such fatty acids were composed of 18 methyleicosanoic acid (18 MEA) and known to be bound to proteins by ester or thioester bond in living or keratinized cells [76, 77]. There have been studies on the LL between hair cuticles in hair; it has been known to be present as a CMC composed of a δ layer, core structure relatively light stained, covered with two β layers in sandwich form [36]. It is difficult to observe the LL with the conventional staining method using OsO_4 or RuO_4. OsO_4 cannot reveal lipid component in tissue. On the other hand, RuO_4, which is routinely used to reveal the epidermal lipids cause severe hair damage. So, new fixative (Lee's fixative) to minimize hair injury was designed to observe the LL in the hair [14]. With this technique, it is possible to observe UV-induced CMC lipid damage directly (Fig. 13.2).

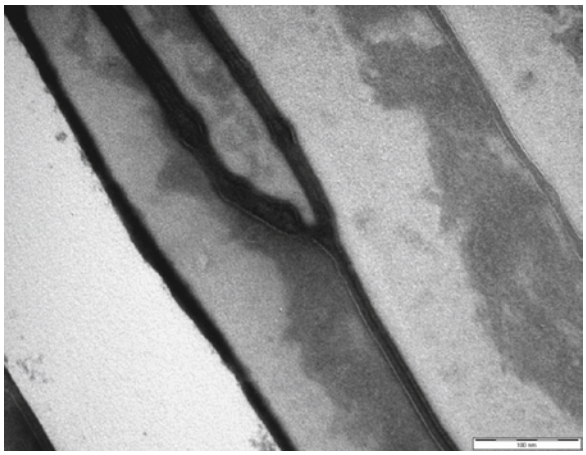

Fig. 13.2 Lipid transmission electron micrograph of hair fiber illustrating electron dense cell membrane complex (CMC) lipid. Note the several bulging areas representing photodamage along the CMC with UVA irradiation

The intercellular lipid of keratinized fibers, together with globular proteins, forms a CMC that connects the keratinous cuticle and cortex cells to a uniform tissue complex. The cell membrane lipids are very important as they make possible a continuous pathway of diffusion into the fiber [43, 80]. Furthermore, according to recent investigation on human hair, a correlation exists between the amount of IL and the moisture content in the hair [51]. A reduction of the amount of lipids by solvent extraction [47] and oxidative damage during chlorine bleaching of wool [21] or during permanent waving of hair [22] favor the diffusion of foreign materials into the fiber [70]. In wool research, important indications of the photochemical lability of IL have been published [81, 82].

Hoting et al. [26] have shown that the CMC lipids of hair fibers are degraded more by VIS light but also by UVA and by UVB light, helping to explain the weakening of the CMC and the multiple step fractures observed in hair exposed to light radiation.

IL from blond and black hair contains the same lipid fractions in comparable amounts. Irradiation with sunlight degrades the IL from blond hair more than those from black hair. UVB and UVA destroy approximately 25% of the cholesterol in blond hair. VIS and global radiation even degrade 60% and more of the initial cholesterol content. In contrast, the cholesterol fraction from black hair is not significantly altered by UVB, UVA, and VIS; in contrast with blond hair, global radiation leads only to a lower extent (24%) of photooxidation of cholesterol in black hair. The photochemical degradation of FFA occurs on blond and black hair by the influence of UVB and UVA to comparable degrees. UVB irradiation reduces the FFA amount by approximately 40% and UVA irradiation by approximately 20%. Differences as a function of the type of pigmentation can be detected for hair irradiated with VIS. The FFA fraction from blond hair was reduced by only 23%. With the exception of the FFA fraction from black hair, IR irradiation does not show a significant degradation of lipids. Global irradiation causes in blond hair a degradation of fatty acids of 33% and in black hair of 42% [26]. The lower photooxidative degradation in black hair suggests that eumelanin, the pigment in black hair, is responsible for this effect. It protects the IL mainly from the photochemical influence of the VIS range of sunlight. The protective function of melanin against photodegradation also applies to the UVB nd UVA ranges.

Take Home Pearls

- As for physical changes of photodamaged hair, dryness, reduced strength, rough surface texture, loss of color, decreased luster, stiffness, and brittleness may occur.
- UV light and oxygen affect not only the melanins but also the amino acids and lipids in the hair and on the cuticle.
- Several amino acids of hair absorb light in this region, and these amino acids are the most subject to degradation by light. The following amino acids have been shown to be degraded by light radiation: cystine and methionine; phenylalanine, tryptophan, and histidine, proline; and leucine.
- The photochemical effects on hair color are strongly dependent on the presence of melanins and chromophores in the hair. Eumelanin is much light stable and provides a better photoprotective effect on hair than pheomelanin.
- Melanin can attribute photoprotection to hair protein, but only in the cortex. As dark hair has more photosensible proteins than light hair, they can show a greater protein loss than light hair. In the cortex, even though dark hair has more photosensible proteins than light hair, they also have more melanin to absorb the UV radiation.

- The hair coloring dyes exhibit different sensitivities to the UVA, UVB, VIS light, and IR portions of solar radiation. VIS light and UVA are mostly responsible for the photofading of artificial hair color.
- ILs of hair fibers are degraded by UV light as well as by VIS light, causing weakening of the CMC exposed to light radiation. Eumelanin is responsible for protecting the integral hair lipid from the photochemical influence of the sunlight.

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Effect of Tobacco Smoking on Hair

14

Ralph M. Trüeb

Core Messages

› Besides being the single most preventable cause of significant morbidity and an important cause of death in the general population, tobacco smoking has been linked to adverse dermatologic effects. A population-based cross-sectional survey has recently demonstrated significant positive associations between androgenetic alopecia and smoking status in men. The mechanisms by which smoking may cause hair loss are multifactorial. The fact that cigarette smoke-associated hair loss is of the androgenetic type indicates that genetic factors contribute, with variances between individuals resulting from different patterns of conduct. In view of the psychological impact of alopecia, increasing public awareness of the association between smoking and hair loss offers an opportunity for health education against smoking.

14.1 Faces Going Up in Smoke

Besides being the single most preventable cause of significant cardiovascular and pulmonary morbidity and an important cause of death in the general population, tobacco smoking has been associated with various adverse effects on the skin [34]. Premature skin ageing has attracted the attention of the medical community only since the 1960s [8, 10, 12, 15, 18, 20, 26, 43, 45], although a relation between smoking and skin complexion was first suggested as early as 1856 [35].

The facial features, designated "smoker's face", were originally defined by Model [26] as one or more of the following: (a) wrinkles typically radiating at right angles from the upper and lower lips or corners of the eyes ("crow's feet"), deep lines on the cheeks, or numerous shallow lines on the cheeks and lower jaw, (b) a gauntness of facial features with prominence of the underlying bony contours, in some cases associated with a leathery, worn, or rugged appearance, (c) an atrophic, slightly pigmented gray appearance of the skin, and (d) a plethoric, slightly orange, purple, and red complexion different form the purply blue colour of cyanosis or the bloated appearance associated with alcoholism. In a survey of 116 patients attending a general medical outpatient clinic (for other than dermatologic conditions), Model found that "smoker's face" was present in 46% of current smokers, whereas 8% of past smokers and no non-smokers had "smoker's face". Others have also addressed the topic of smoking as a risk factor for facial wrinkling and come to similar conclusions [10, 12, 15, 20, 45].

The mechanisms by which smoking causes wrinkling are believed to be multifactorial, and related to effects of cigarette smoke on the microvasculature [41], cutaneous collagen [32], and elastic tissue [23], to pro-oxidant effects of smoking [19, 42], and increased hydroxylation

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of estradiol [13], as well as inhibition of the enzyme aromatase [28], which converts androgens to estrogens, creating a relative hypoestrogenic state. Cigarette smoke has been shown to increase plasma neutrophil elastase activity [44], which may also contribute to abnormal skin elastin, and matrix metalloproteinase (MMP)-1 (collagenase) expression was shown to be increased in skin of smokers [22], which may contribute to degradation of collagen. Expression of both MMP-1 and MMP-3 have been shown to be induced in cultured human fibroblasts in a dose-related manner by cigarette smoke extracts, while the expression of tissue inhibitors of metalloproteinases (TIMPPs) were not influenced [46]. The fact that not all smokers develop a "smoker's face" may indicate that genetic factors may also contribute.

The histologic features associated with smoking have not been studied as extensively, but have demonstrated increased elastosis [3, 14].

Given the public's overwhelming quest for a young and pleasing appearance, the consensus is that education about the link between cigarette smoking and skin ageing could motivate young people not to smoke or to quit smoking [11].

14.2 Smoking Out the Hair

Alike the skin, the hair is subject to intrinsic or physiologic ageing, and extrinsic or accelerated ageing due to external factors [40]. Intrinsic factors are related to individual genetic and epigenetic mechanisms with interindividual variations. Prototypes are familial premature graying, and androgenetic alopecia. Extrinsic factors include exposure to tobacco smoke.

In 1996, Mosley and Gibbs [27] reported in patients visiting a general surgical outpatient clinic in the United Kingdom for the first time a significant relationship between smoking and premature gray hair in both men and women, and between smoking and baldness in men. Since the number of alopecia in women was very small, no corresponding calculation could be carried out for hair loss in women.

Subsequently, the observation of strikingly dissimilar androgenetic alopecia in a 52-year-old monozygotic male twin pair led to further speculations on the possibility of an association between smoking and hair loss [38], since studies on the degree of alopecia among monozygotic twins aged over 50 have shown that intrapair differences are negligible in 92%, slight in 8%, and striking in none [17]. A salient feature differentiating the twin brothers in their personal histories was that the balding brother admitted to long-standing, heavy cigarette smoking, while the other was a non-smoker. A succeeding study failed to confirm this association [33].

Eventually, a recent population-based cross-sectional survey among Asian men 40 years or older showed statistically significant positive associations between moderate or severe androgenetic alopecia and smoking status, current cigarette smoking of 20 cigarettes or more per day, and smoking intensity [36]. The odds ratio of early-onset history for androgenetic alopecia grades increased in a dose-response pattern. Risk for moderate or severe androgenetic increased for family history of first-degree and second-degree relatives, as well as for paternal relatives. A reservation to be made is the role of negative psychological effects of androgenetic alopecia on self-esteem and peculiarities of psychological adjustment. Although in this study data were collected with respect to age at onset of androgenetic alopecia, and age at start of smoking, there is no information on the temporal relationship of these with respect to the question, whether smoking contributes to the development of androgenetic alopecia, or alternatively represents a form of behavioural coping to the impairment in physical appearance resulting from alopecia.

The mechanisms by which smoking accelerates hair loss have not been examined, but it is likely that they are similar to the effects on the skin: The cutaneous microvasculature is constricted by acute and long-term smoking. Evidence of the consequence of impaired circulation and wound healing is a higher complication rate of hair restoration surgery in smokers vs. non-smokers [9].

Besides inducing local ischemia, the decreased capillary blood flow in the dermal papilla of the hair follicles may focally shunt more toxic substances. Cigarette smoke contains over 4,800 chemicals, many of which are known to be toxic to cells and 69 are known to cause cancer. Apart from cancer, smoking is also a major risk factor in chronic obstructive pulmonary disease, heart disease and cerebrovascular insult, and other disorders such as slowed healing of wounds, impotence, infertility and peptic ulcer disease. Smoke genotoxicants metabolised in hair follicle cells may cause DNA damage through the production of DNA adducts, and smoking-associated mitochondrial DNA mutations have been shown in human hair follicles [24], though the relevance of these for hair follicle pathology is as yet unknown.

Both nicotine and cotinine is detected in hair samples of smokers [16]. The detection of nicotine even in hair samples from ancient Egyption mummies [2] has caused some confusion in the scientific community, since these findings were immediately identified as improbable on the ground that nicotine was known to be derived only from the American plant *Nicotiana tabacum*, and the suggestion that such a compound could have found its way to Egypt before Columbus' discovery of America in 1492 seemed patently impossible. Nicotine is named after Jean Nicot, sieur de Villemain (1530–1604), French ambassador in Portugal, who sent tobacco and seeds from Brazil to Paris in 1560 significantly contributing to the spread and use of tobacco in the Old World.

Since substantial extracellular matrix remodelling is involved in the hair follicle growth cycle, especially during catagen-associated hair follicle regression [29], it is conceivable that cigarette smoke-induced imbalance in the intra- and perifollicular protease/antiprotease systems controlling tissue remodelling may also affect the hair follicle growth cycle.

Finally, smoking-induced oxidative stress and a disequilibrium of anti-oxidant systems may lead to the release of pro-inflammatory cytokines from follicular keratinocytes, which by themselves have been shown to inhibit the growth of isolated hair follicles in culture [30]. Moreover, adjacent fibroblasts are fully equipped to respond to such a pro-inflammatory signal. On the occasion that the causal agent persists, sustained micro-inflammation of the hair follicle is the result, together with connective tissue remodelling, where again collagenases play an active role, and are believed to contribute to perifollicular fibrosis [25].

Possibly the smoking-associated premature graying of hair, observed by Mosley and Gibbs [27], could be related to the oxidative stress as well. Ultrastructural studies of graying hair have shown that melanocytes contain melanosomes packaged within autophagolysosomes. The removal of melanosomes into autophagolyosomes suggests that they are defective, possibly with reactive melanin metabolites. This interpretation is supported by the observation that melanocytes in graying hair bulbs are frequently highly vacuolated, a common cellular response to increased oxidative stress. The extraordinary melanogenic activity of pigmented bulbar melanocytes, continuing for up to 10 years in some hair follicles, is likely to generate large amounts of reactive oxygen species via the hydroxylation of tyrosine and the oxidation of DOPA to melanin. If not adequately removed by an efficient anti-oxidant system, an accumulation of these reactive oxidative species will generate significant oxidative stress. It is possible that the anti-oxidant system becomes impaired with age leading to damage to the melanocyte itself from its own melanogenesis-related oxidative stress (intrinsic ageing) [37], while additional oxidative stress from smoking could accelerate this process (extrinsic ageing).

Interestingly, a recent experiment demonstrated that C57BL/6 mice exposed to cigarette smoke developed hair loss, while no alopecia occurred in sham-exposed mice. Smoke-exposed mice had extensive atrophy of the epidermis, reduced thickness of the subcutaneous tissue, and scarcity of hair follicles with massive apoptosis in the hair bulbs at the edge of alopecic areas [6]. This effect was prevented by the oral administration of *N*-acetylcysteine, an analogue and precursor of L-cysteine and reduced glutathione, as well as by L-cystine, the oxidised form of L-cysteine, which is a key hair component, in combination with vitamin B6, which plays a role in L-cystine incorporation in hair cells [7]. The effect may be related to the glutathione-related detoxification system.

The fact that cigarette smoke-associated hair loss is of the androgenetic type, indicates that genetic factors contribute. On the other hand, a local relative hypoestrogenic state induced by cigarette smoking due to increased hydroxylation of estradiol and inhibition of the enzyme aromatase may also contribute to an increase of androgen-dependent hair thinning [21].

Finally, variances between individuals also may result from patterns of conduct, in as much as persons exposed to one risk factor (smoking) are often exposed to others as well, such as intake of androgens or their precursor (such as DHEA), or of progestins with androgenic activity (in oral contraceptives and hormonal replacement therapy), excessive ultraviolet light exposure [39], and stress [1], all of which have been implicated in the one way or other in the pathogenesis of alopecia and associated conditions of the scalp.

14.3 Another Opportunity for Health Education Against Smoking?

The appearance of hair plays an important role in people's overall physical appearance and self-perception. The well-recognised psychological effects of androgenetic

Fig. 14.1 Increasing public awareness to the negative effects of cigarette smoking on hair (turn-off tactic: social stigma). Poster from: Media Awareness Network (www.media-awareness.ca)

alopecia on affected men [4] and women [5], and our society's veneration of youth and its attributes, seem to offer a good opportunity for prevention or cessation of smoking (Fig. 14.1). Increasing public awareness of the association between smoking and hair loss would seem more effective than the link between smoking and facial wrinkles, since the latter can be effectively counteracted by current cosmetic dermatologic procedures, while treatment options for androgenetic alopecia have remained limited and are as yet less than satisfactory [31].

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Age-Related General Problems Affecting the Condition of Hair

15

Ralph M. Trüeb

Core Messages

› Older patients suffer from a variety of conditions that affect the hair, i.e. nutritional deficiency, endocrine disorders, psychologic problems, and drug-related adverse effects. The quantity and quality of hair are closely related to the nutritional, general, and mental health state of the individual. Sometimes, symptoms of overt pathologic conditions are misinterpreted as signs of normal aging, ignored and left untreated. In taking care of older patients with hair problems, it is therefore important to be suspicious of the possibility of a more general problem underlying the patient's complaint. The increasing number of chronic conditions per patient and the increasing amount of multimorbidity in the elderly population lead to a more complex approach to successful therapy of hair problems in the elderly.

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15.1 Aging Hair

The appearance of hair plays an important role in people's overall physical appearance and self-perception. With today's increasing life expectations, the desire to look youthful plays a bigger role than ever. Though the pharmaceutical and hair care industry have become aware of this consumer demand and are delivering active products for the maintenance of healthy and beautiful hair, there remains a number of age-related general problems affecting the condition of hair that need to be addressed as well.Aging hair is characterized by loss of follicle rigor, consistent with age-dependent slowing of other body activities, failure to pigment the hair shaft (graying), decrease in hair growth, and reduction in the diameter of the hair shaft. The condition of the hair depends on hereditary and ethnic factors, condition of the scalp, hair care and styling habits, external factors and hair damage, and nutritional and overall health status. Older patients suffer from a variety of conditions that affect the hair: nutritional deficiency, endocrine disorders, psychologic problems, and drug-related adverse effects. Sometimes, symptoms of overt pathologic conditions are misinterpreted as signs of normal aging, ignored and left untreated. In taking care of older patients with hair problems, it is therefore important to be suspicious of the possibility of a more general problem underlying the patient's complaint.

It is the objective of this chapter to focus on age-related general problems affecting the condition of the hair.

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15.2 Rare Premature Aging Syndromes

Scientists are particularly interested in the premature aging syndromes, because these might reveal clues about the normal process of aging. Hutchinson–Gilford progeria and Werner's syndrome are repeatedly given as examples of premature aging and as evidences for the genetic basis of aging. However, the analogy of these syndromes with physiologic aging has been challenged. These pathologic conditions should rather be viewed as deviations from normal development [48]. Since the mesenchymal tissue is severely affected in both diseases, and the inductive phenomena necessary for the differentiation and development of the different organs take place through the interaction of connective tissue with other tissues, it is conceivable that defects in the connective tissue lead to a variety of deviations in the whole organism [13]. In fact, both syndromes have been classified with the very large group of idiopathic dwarfism, which includes Cockayne, Bloom, and Rothmund-Thomson syndromes [35]. Indeed, Werner's syndrome patients have reduced growth hormone (GH) secretion and reduced levels of both GH and insulin-like growth factor-1 [66].

There are many significant differences between the normal aging process and the manifestations of both Hutchinson–Gilford progeria and Werner's syndrome. Because these "accelerated aging" diseases display different aspects of aging, but never every aspect, they are often called "segmental progerias." Some biogerontologists question that such a thing as "accelerated aging" actually exists, at least partly on the grounds that all of the so-called accelerated aging diseases are segmental progerias. It should be added that there are no neuronal changes in both Hutchinson–Gilford progeria and Werner's syndrome such as senile plaques, neurofibrillar tangles, granulovacuolar degeneration, and there is no increase in lipofuscin and amyoid [51]. Symptoms of "accelerated aging" of the hair include premature graying and premature loss of hair. The loss of hair is more generalized than in normal aging.

15.2.1 Hutchinson–Gilford Progeria

Hutchinson–Gilford progeria is an extremely rare condition in childhood where symptoms resembling some aspects of aging are manifested at an early age. It is a genetic disorder caused by mutations in one of the major architectural proteins of the cell nucleus, Lamin A protein. Nuclear Lamin A is a protein scaffold on the inner edge of the nucleus that helps organize nuclear processes such as RNA and DNA synthesis. The earliest symptoms include failure to thrive and a scleroderma-like skin condition. As the child ages past infancy, additional conditions become apparent: alopecia, wrinkled skin, and a distinctive appearance with small face and jaw and pinched nose. Later, the condition causes atherosclerosis and cardiovascular problems. At least 90% of patients die from heart attacks or strokes. Affected children have small, fragile bodies like those of elderly people. The development of symptoms is comparable with aging at a rate six to eight times faster than normal, although certain age-related conditions do not occur. Specifically, patients show no neurodegeneration or cancer predisposition, and do not develop "wear and tear" conditions associated with aging, like cataracts and osteoarthritis. Few with progeria live beyond 13 years of age.

15.2.2 Werner's Syndrome

Werner's syndrome is another very rare genetic disorder characterized by the appearance of premature aging. It more closely resembles accelerated aging than any other segmental progeria. The defect is on a gene that codes DNA helicase, and directly caused by shorter-than-normal length telomere maintenance. As a result, DNA replication is impaired. Individuals with this syndrome typically develop normally until they reach puberty. An early sign is the lack of a teenage growth spurt, which results in short stature. Following puberty they age rapidly, so that by age 40 they often appear several decades older. When affected individuals are in their twenties or thirties, other symptoms appear and include premature loss and graying of hair, hoarseness of the voice, and scleroderma-like thickening of the skin. Affected individuals typically have a characteristic facial appearance described as "bird-like" (Fig. 15.1). Patients with Werner's syndrome also exhibit genomic instability, hypogonadism, and various age-associated disorders, including: diabetes mellitus, atherosclerosis, heart disease, cataracts, and cancer. People affected by Werner syndrome usually do not live past their early fifties, often dying from heart disease or cancer.

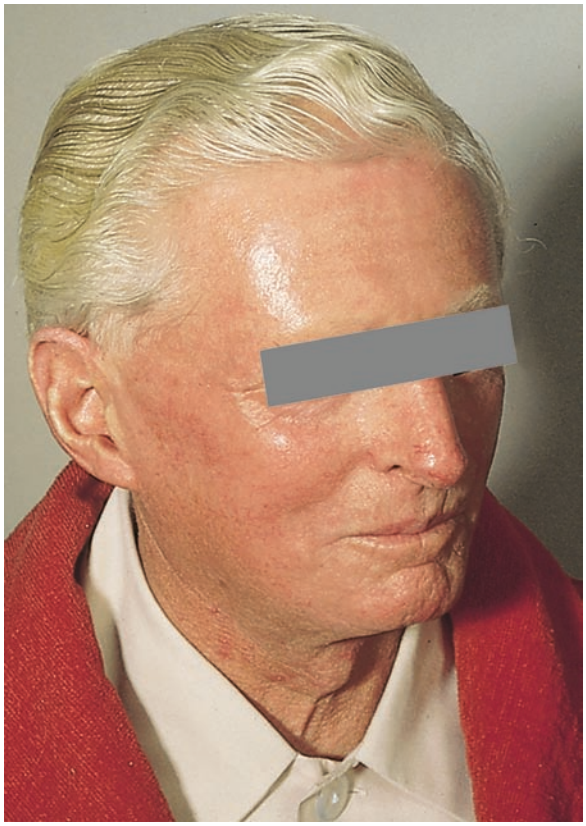

Fig. 15.1 Werner's syndrome: premature graying with characteristic face

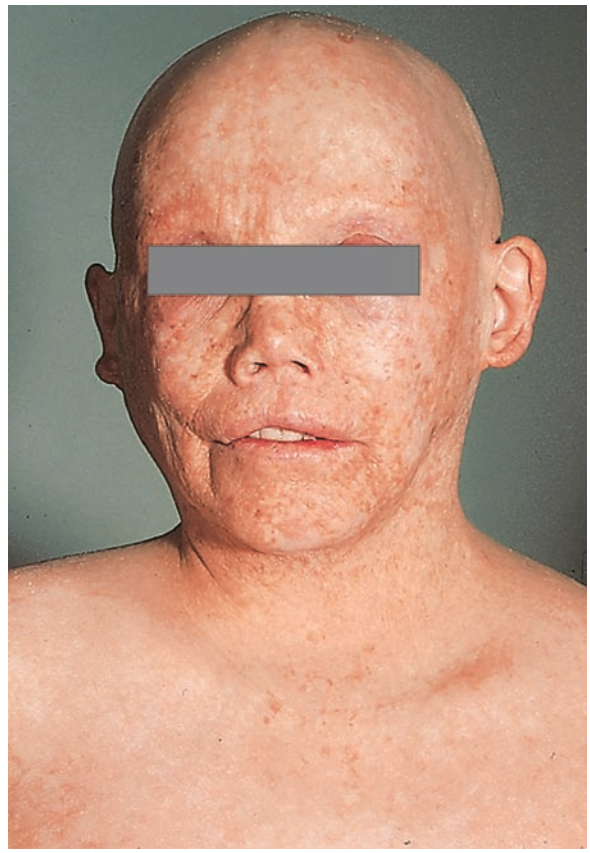

Fig. 15.2 Rothmund–Thomson syndrome: total alopecia with saddle nose and poikiloderma

15.2.3 Rothmund–Thomson Syndrome

Rothmund–Thomson syndrome is yet another rare hereditary condition in which mutations in a DNA helicase gene (RECQL4) has been implicated. Symptoms are poikiloderma, juvenile cataracts, saddle nose, short stature, congenital bone defects, particularly radial ray anomalies, alopecia (Fig. 15.2), and hypodontia. There have been several reported cases associated with osteosarcoma.

15.3 Nutritional Deficiency in the Elderly

The quantity and quality of hair are closely related to the nutritional state of an individual. Normal supply, uptake, and transport of proteins, calories, trace elements, and vitamins are of fundamental importance in tissues with a high biosynthetic activity such as the hair follicle. Because hair shaft is composed almost entirely of protein, protein component of diet is critical for the production of normal healthy hair. The rate of mitosis is sensitive to the calorific value of diet, provided mainly by carbohydrates stored as glycogen in the outer hair root sheath of the follicle. Finally, a sufficient supply of vitamins and trace metals is essential for the biosynthetic and energetic metabolism of the follicle. In instances of protein and calorie malnutrition, deficiencies of essential amino acids, of trace elements, and of vitamins occur, and hair growth and pigmentation may be impaired. The effects of nutrition on hair growth have been recognized from observations in inborn errors of metabolism, in deficiency disorders, and from supplementation studies in animals and humans.

It appears that on a typical Western diet, the hair follicle should have no problem in producing an appropriate hair shaft. Nevertheless, vitamin and nutritional

deficiencies are common in the elderly population. As many as 50% have a vitamin and mineral intake less than the recommended dietary allowance, and as many as 30% of the elderly population have subnormal levels of vitamins and minerals [40].

In general, malnutrition is due to one or more of the following factors: inadequate food intake, food choices that lead to dietary deficiencies, and illness that causes increased nutrient requirements, increased nutrient loss, poor nutrient absorption, or a combination of these factors. Nutritional inadequacy in the elderly can be the result of one or more of the following factors:

A physiologic decline in food intake is seen in people as they age, regardless of their illness, and is often referred to as "anorexia of aging" It probably involves alterations in neurotransmitters and hormones that affect the central feeding drive and the peripheral satiation system. Moreover, sensory decline in both olfaction and taste decreases the enjoyment of food and dietary variety.

Socioeconomic status and functional ability have an important influence on nutritional status. When financial concerns are present, meals are skipped and food that is purchased may not provide a nutritionally adequate diet. Declines in both physical and cognitive functional status affect an individual's ability to shop for food and to prepare meals. Nutritional problems are further compromised by social isolation, which commonly leads to apathy about food and decreased intake.

The older person has experienced change and loss through retirement, disability, and death of friends and family, as well as change in financial, social, and physical health status. These changes may lead to depression. Depression is often unrecognized in older individuals. Malnutrition may be a presenting symptom of depression in the elderly.

Ultimately, underlying pathology and medical treatment can cause anorexia and malnutrition. Problems with dentition and disorders of the gastrointestinal system are related to poor intake and malabsorption of nutrients. Many diseases (i.e., thyroid, cardiovascular, and pulmonary disease) lead to an increased metabolic demand, and at the same time decreased appetite and caloric intake. Drugs may affect nutritional status through side effects (i.e., anorexia, nausea, and altered taste perception) and through alteration of nutrient absorption, metabolism, and excretion.

15.3.1 Protein-Calorie Malnutrition

Protein-calorie malnutrition is probably the best studied of possible forms of undernutrition in man. It occurs commonly in developing countries; in developed countries, it predominantly affects chronically ill and hospitalized patients: children on protein restricted diets for management of urea-cycle disorders, such as argininosuccinic aciduria; infants on milk-restricted diets because of suspected lactose intolerance or milk protein allergy; children and adolescents with gastrointestinal disease, cystic fibrosis, and anorexia nevosa, and patients with chronic renal failure, severe neurologic impairment, or malignancy. *Kwashiorkor* (Ghanian for "copperhead") is due to a deficit of protein in an otherwise calorically adequate diet. Alterations in hair color and texture are: short and dry hair, with increased cuticular weathering, reddish-brown to rusty hair color, and a raised telogen count and atrophy of anagen bulbs. Other symptoms include anemia, (hypoproteinemic) edema, and hypopigmentation of skin with dry, fissured areas, and cracked appearance ("flaky paint"). *Marasmus* is due to a deficit of calories exceeding the deficit of protein. The amino acids are utilized to maintain body function. The result is an atrophy of most organs; the hair is thin, sparse, and easily plucked.

15.3.2 Biotin Deficiency

Dietary deficiency of biotin is rare, since biotin is synthesized by intestinal bacteria. Recommended dietary allowances are not established. Deficiency has been reported in association with parenteral alimentation, altered intestinal flora (antibiotic treatment), and excessive ingestion of raw egg white (containing avidin that binds biotin). Symptoms of biotin deficiency are alopecia in combination with periorificial dermatitis, blepharoconjunctivitis, and recurrent infections.

15.3.3 Vitamin C Deficiency (Scurvy)

Vitamin C or ascorbic acid is not synthesized in the human body; therefore, dietary intake is essential. The recommended dietary allowance for adults is

60 mg/day. Vitamin C deficiency or *scurvy* is mainly found in alcoholics and elderly living alone. Patients with chronic disease, such as cancer or chronic renal failure are also at risk. Mucocutaneous symptoms of vitamin C deficiency are related to the role of vitamin C in collagen synthesis: ecchymoses, bleeding gums, follicular hyperkeratosis with corkscrew hairs, and perifollicular hemorrhages. The follicular changes are a direct consequence of decreased cross-linkage of hair keratin resulting from decreased number of reduced disulfide bonds and curling of follicles resulting from altered perifollicular connective tissue. Hemorrhages, poor wound healing, and infections are significant causes of morbidity and death. Adult scurvy can be treated with 300–1,000 mg of ascorbic acid per day, and improvement is generally seen in less than a week.

15.3.4 Vitamin B12 Deficiency (Pernicious Anemia)

Deficiency of vitamin B12 is seen in strict vegetarianism, celiac disease, stagnant bowel syndrome, pancreatic disease with steatorrhea, ingestion of fish tapeworm, and atrophic gastritis with antibodies to intrinsic factor (pernicious anemia). Clinical manifestations are: megaloblastic anemia, peripheral neuropathy, and degeneration of the posterior and lateral spinal cord. Mucocutaneous signs include atrophic glossitis (Hunter, Fig. 15.3), angular cheilitis (Fig. 15.4), and premature graying. The peripheral neuropathy may increase the incidence of trauma. The recommended daily intake of vitamin B12 is 3 mg.

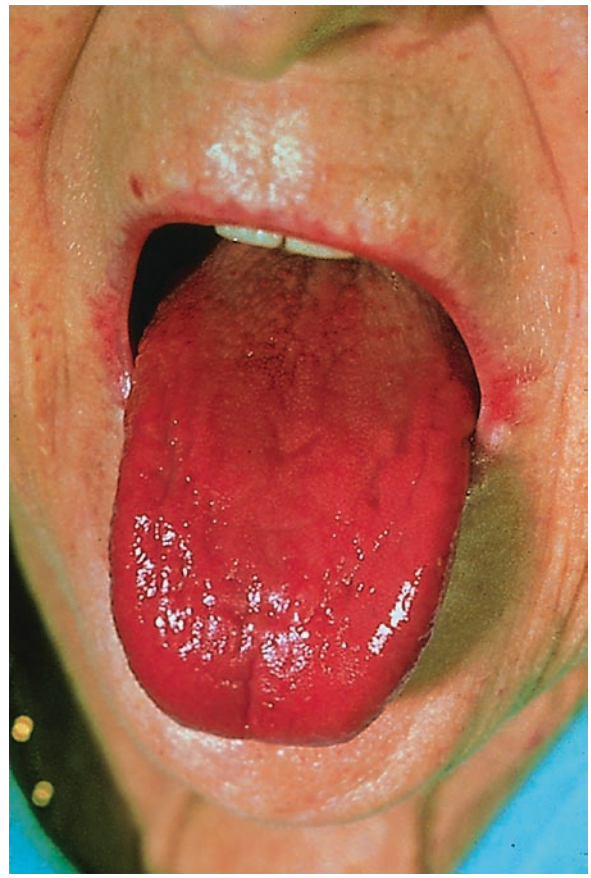

Fig. 15.3 Clue to vitamin B12 deficiency: Hunter's glossitis

15.3.5 Niacin Deficiency (Pellagra)

Pellagra (from: "pelle agro"= rough skin) results from a deficiency of the B vitamins, most notably niacin or vitamin B3. It occurs endemically in areas where maize and millet form the main diet (Asia, Africa, India); sporadic cases are seen in individuals with inadequate dietary intake (alcoholics, anorexia nervosa), impaired absorption of niacin (Crohn's disease), drugs that interfere with niacin metabolism (INH), and tumors that interfere with niacin metabolism (carcinoid). Symptoms are a peculiar photosensitive dermatitis with hyperpigmentation (Figs. 15.5 and 15.6), diarrhea, dementia, and ultimately death ("4D's"). Initial manifestations are nonspecific and include anorexia, weakness, irritability, weight loss, mouth soreness, glossitis, stomatitis, and

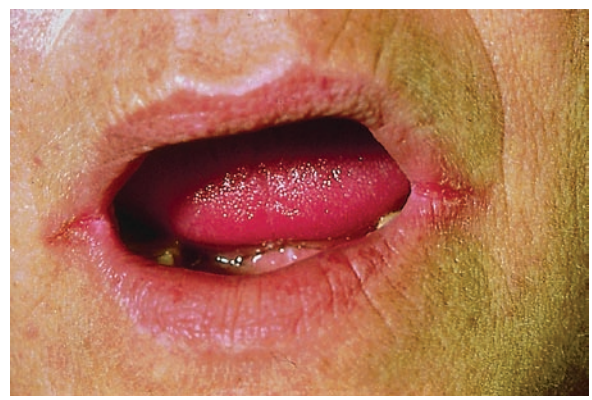

Fig. 15.4 Clue to vitamin B12 deficiency: angular cheilitis

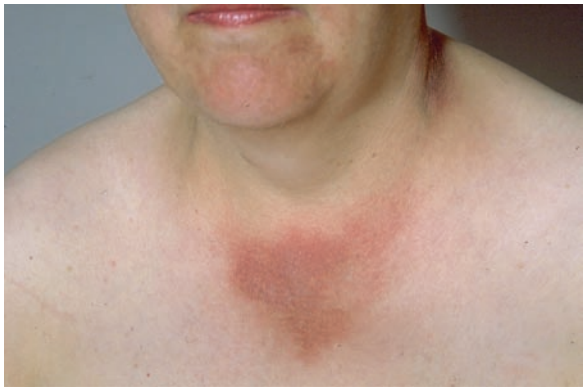

Fig. 15.5 Niacin deficiency: pellagra

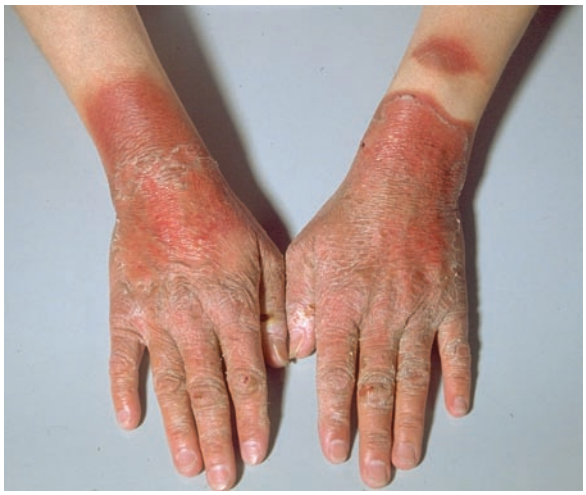

Fig. 15.6 Niacin deficiency: pellagra

diffuse hair loss. Niacin is an essential component of NADH that connects the citric acid cycle to the process of oxidative phosphorylation important for the generation of ATP and therefore energy supply. The recommended daily intake is 6.6 mg/1,000 kcal, and at least 13 mg/day.

15.3.6 Deficiency of Essential Fatty Acids

Deficiency of the long-chain polyunsaturated fatty acids linoleic acid and alpha-linolenic acid may be due to impaired fat absorption in children with biliary atresia, cystic fibrosis, or intestinal lymphangiectasia, dietary supplements consisting primarily of medium-chain triglycerides in short-bowel syndrome, and inadequately supplemented parenteral alimenation. Cutaneous manifestations of deficiency of essential fatty acids are severe dryness and scaling of skin, redness and scaling of scalp and eyebrows, weeping intertriginous lesions, the hair becoming dry, unruly, lighter in color, and a significant telogen hair shedding.

15.3.7 Iron Deficiency

Iron deficiency represents the most common nutritional deficiency with the highest prevalence in adolescent girls and women of childbearing age. Nevertheless, the prevalence of iron deficiency is 6–9% in women with 50 years of age and older in the USA [19]. Whereas the most common causes of iron deficiency are menstrual blood loss, pregnancy, and lactation in premenopausal women, in postmenopausal women they are decreased absorption and gastrointestinal bleeding.

Total body iron is distributed among storage iron, transport iron, and functional iron. Storage iron is the body's iron reserves that are tissue bound and measured by serum ferritin concentration, transport iron is transported to the tissues and measured by transferrin concentration and saturation, and functional iron consists of iron that is bound to hemoglobin, myoglobin, and diverse enzymes. It is measured by hemoglobin concentration.

Iron deficiency is viewed as a continuum ranging from iron depletion to iron deficiency anemia. In the former, body iron stores are reduced, but functional and transport iron remain normal, leaving little reserves if the body requires more iron; in the latter, storage, transport, and functional iron are severely decreased and can lead to impaired function of multiple organ sites.

The symptoms of iron deficiency include fatigue and decreased exercise tolerance; signs of severe anemia include skin and conjunctival pallor, tachycardia, and low blood pressure; dermatologic findings include hair loss (telogen effluvium), cheilosis, and koilonychia (Fig. 15.7). It must be noted, however, that some patients with iron deficiency and even anemia may remain completely asymptomatic. Several studies have evaluated the relationship between iron deficiency and hair loss. Almost all of these studies have focused

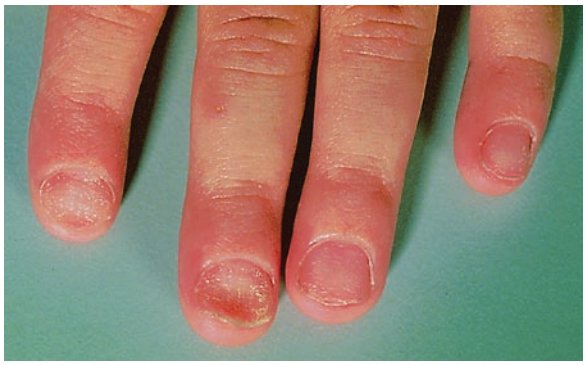

Fig. 15.7 Clue to iron deficiency: koilonychia

exclusively on women. Although nonanemic iron deficiency as an etiologic factor for diffuse hair loss in women was first postulated by Hard [36], it is not until recently that the significance of iron stores as assessed by serum ferritin levels in women with hair loss has been systematically studied [79]. The various observational studies that evaluated the association between decreased ferritin levels and hair loss have resulted in opposing conclusions [4, 41, 67–70, 75]. The controversy starts with a debate over what is the normal serum ferritin level for women [84], and is further complicated by the use of different reference ranges by different laboratories, based on individual interpretations of the literature on this subject. A cutoff point of 10–15 μg/L is considered to yield a sensitivity of 59% and a specificity of 99% for diagnosing iron deficiency [74] and is used by many laboratories as the lower limits of normal based on reference sample groups. In women of child-bearing age, using a cut off of 10–15 μg/L yields a sensitivity of 75% and specificity of 98% [18]. A cut off of 30 μg/L yields a sensitivity of 92% and a specificity of 98% [74].

To evaluate the relationship between serum ferritin levels and hair loss activity determined by trichograms, we performed a retrospective case study of 181 women with hair loss who underwent biochemical investigations and trichograms. The age range of the women was between 13 and 81 years (mean: 42).A ferritin level of > 30 μg/L is seen in 61.9%, between 10 and 30 μg/L in 30.4%, and ≤ 10 μg/L in 7.7%. There was a significant correlation between the age of women and serum ferritin levels, with younger (menstruating) women having lower ferritin levels. No correlation was found between ferritin levels > 10 μg/L and telogen rates. We concluded that in women with hair loss, the role of tissue iron status within limits regarded as normal has probably been overestimated, since a majority of otherwise healthy women with hair loss had ferritin levels > 30 μg/L (cut off with 92% sensitivity and 98% specificity for iron deficiency), and no correlation was found between hair loss activity and ferritin levels > 10 μg/L [11].

Finally, a caveat should be spoken against uncritical iron supplementation, since there is a possibility that increased iron storage could enhance DNA oxidative injury by inducing the Fenton reaction [90].

15.3.8 Zinc Deficiency (Acrodermatitis Enteropathica)

In children, zinc deficiency is a rare autosomal recessive genetic disorder with absorption of zinc resulting in a condition called acrodermatitis enteropathica. It manifests at the time of weaning, and is associated with reduced absorption of unsaturated fatty acids and impaired desaturation of linoleic and alpha-linolenic acids, which in part explains some of the cutaneous manifestations. These are a characteristic psoriasiform acral and periorifical dermatitis with typical histology, susceptibility to infection, superinfection of skin lesions with *Candida albicans* and *Staph. aureus*, stomatitis, angular cheilitis, blepharoconjunctivitis, nail dystrophy, alopecia, and sparse, white, thin, and brittle hair (Fig. 15.8). In adults and the elderly, the manifestations of acquired zinc deficiency are similar. Intake of 8–10 mg of zinc per day is adequate and obtained in a normal

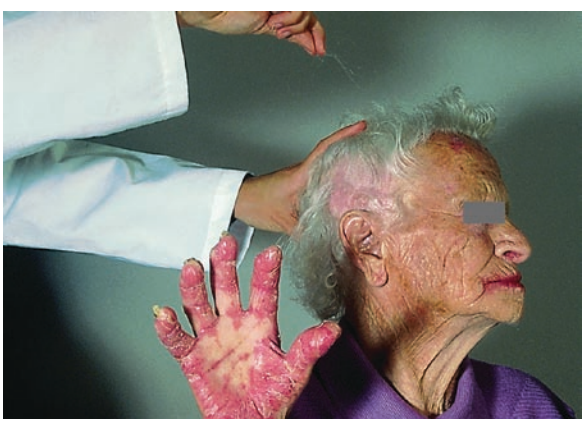

Fig. 15.8 Acrodermatitis enteropathica (zinc deficiency): pathologic pull test and acral dermatitis

diet. Zinc deficiency is seen in chronic alcoholism, anorexia nervosa, kidney disease, or with impaired absorption of zinc due to: diet with high phytate content, drugs that chelate zinc (ACE inhibitors), pancreatitis, or gastrointestinal bypass surgery.

15.3.9 Copper Deficiency

In children, copper deficiency is again a rare autosomal recessive genetic disorder with intestinal malabsorption of copper resulting in markedly decreased copper levels in brain, liver, serum, and hair, and dysfunction of a number of copper-dependent enzymes including ascorbic acid oxidase and tyrosinase. Clinical manifestations begin in infancy with degeneration of brain, bone, and connective tissue, expressionless face, with pale, lax skin and plump cheeks, hypopigmented hair, and a characteristic hair shaft anomaly (pili torti, Menkes' kinky hair syndrome). Copper in hair is essential for the oxidation of thiol groups to dithio crosslinks essential for resilient properties of keratin fibers. Dietary deficiency in humans is rare. A diet containing 2–3 mg of copper per day is sufficient. Copper deficiency is seen in premature babies, severely malnourished children, with inadequately supplemented parenteral alimentation, and with prolonged oral zinc therapy. Sheep fed on copper deficient pastures produce wool that is weak, shows abnormal dyeing and processing qualities, and lacks grimp. Clinical manifestations of acquired copper deficiency in humans are: microcytic anemia, leucopenia, myelopathy, and hypopigmentation of hair. No other specific hair anomaly has been reported.

15.3.10 Selenium Deficiency

Selenium is covalently bound to cysteine, thereby substituting sulfur in the sulfhydryl groups. It is an essential component of the antioxidant enzyme glutathione peroxidase. Dietary deficiency has been reported in areas where the soil content is poor in selenium, as in Keshan, China (Keshan disease), and in patients receiving total parenteral nutrition. Clinical manifestations of selenium deficiency are cardiomyopathy and muscle pain and weakness with an elevation of transaminase and creatin kinase levels. Cutaneous manifestations are white nails and hypopigmentation of skin and hair (pseudoalbinism).

15.3.11 Amino Acids

Inborn errors of amino acid metabolism that affect the hair are homocystinuria, Hartnup disease, and methionine malabsorption syndrome (Oasthouse disease). Homocystinuria is an autosomal recessive disorder of cystathionin synthetase with the accumulation of homocystine. Patients present with a Marfan-like syndrome, premature arteriosclerosis, fine, sparse, and hypopigmented hair. Hartnup disease is an autosomal recessive disorder of renal and intestinal transport of neutral aminoacids with Pellagra-like syndrome (due to disorder of niacin metabolism), premature graying, and alopecia. Methionine malabsorption syndrome or Oasthouse disease is an autosomal recessive selective intestinal malabsorption of methionin resulting in central nervous system impairment (oligophrenia, seizures) and pseudoalbinism with white hair.

The rationale for the use of a dietary supplement based on the amino acid L-cystine for treatment of hair loss is based on the biochemistry of cystine metabolism, clinical observations in disorders of cystine metabolism and cystine deficiency, and results of animal and human studies.

L-cystine, a natural, aliphatic amino acid, is a constituent of keratin. Accordingly, hair contains a high proportion of cystine (15.9%). Cysteine, formed from cystine, is the limiting factor in the biosynthesis of the natural antioxidant glutathione. In HIV trichopathy, protein calorie malnutrition, together with deficiencies of copper, zinc, selenium, and disorders of cystine-dependent amino acid metabolism and glutathione-dependant detoxification mechanisms have been postulated to underlie dry, fragile hairs, hair loss, and premature graying [71].

In the 1960s, the role of cystine in the production of wool proteins was investigated, and it was found that enrichment of even what appeared to be a normal diet with sulfur-containing amino acids increased wool production in sheep [28, 33, 62]. More recently, it was shown that the noxious effect of cigaret smoke in exposed C57BL/6 mice could be abrogated by the oral administration of *N*-acetylcysteine, an analog and

precursor of L-cysteine and reduced glutathione, as well as by L-cystine, the oxidized form of L-cysteine, which is a key hair component, in combination with vitamin B6, which plays a role in L-cystine incorporation into hair cells [22]. The effect may be related to the glutathione-related detoxification system.

When considering which nutrients could be used for improving hair growth in humans, cystine was therefore a candidate. Since then, mainly in the early 1990s, studies on the effect of dietary supplements containing L-cystine, usually in combination with B-complex vitamins or medicinal yeast that is rich in B-complex vitamins, have been published, exclusively in German language, showing improvements in the trichogram (hair pluck test), in hair swelling as a criterion for hair quality, and in the tensile strength of the hair fiber [2, 14, 59]. We performed a study combining epiluminiscence microscopy with digital image analysis (TrichoScan) to demonstrate that a L-cystine and B-complex vitamin-based nutrient influences hair growth in otherwise healthy women between 25 and 65 years of age with telogen effluvium [43]: after 6 months of treatment the active compound group showed a statistical significant improvement and normalization of the anagen hair rate, whereas the placebo group did not. Improvement of the anagen count has previously also been shown for treatment of hair loss in women with another combination product with L-cystine using the phototrichogram technique [31]. The hair count, hair density, and cumulative hair shaft diameter did not show any changes from the baseline values. Nevertheless, the change in anagen hair rate was sufficient to reflect in clinical outcome, since the appearance of hair growth in the global photographic assessment was judged better, probably owing to the increase of the proportion of actively growing hairs in anagen (Fig. 15.9). The mechanism of action is not known, but these data suggest that the active compound has a therapeutic effect probably owing to an induction of anagen. Since regressive alopecias, such as female pattern hair loss and senescent alopecia, are due to progressive shortening of the anagen phase and miniaturization of the hair follicle, it is apparent that they benefit from treatment with an agent that has an impact on hair count, hair density, and cumulative hair shaft diameter, such as minoxidil. Nevertheless, synchronization phenomena tend to complicate the course of the regressive alopecias, since with a shortened anagen phase, synchronization will tend to be more marked. In this case, it is conceivable that adding a L-cystine and B complex vitamin based nutrient to the treatment regimen may be beneficial. Moreover, it has been shown in hair follicle cultures, that minoxidil not only increases the incorporation of thymidine (as a marker of cell division), but also leads to an increased uptake of cysteine by the hair follicle [15]. Consistent with this, the regression analysis showed that neither the presence of hair thinning at the vertex nor age had influence on changes in anagen hair rate. Finally, serum ferritin

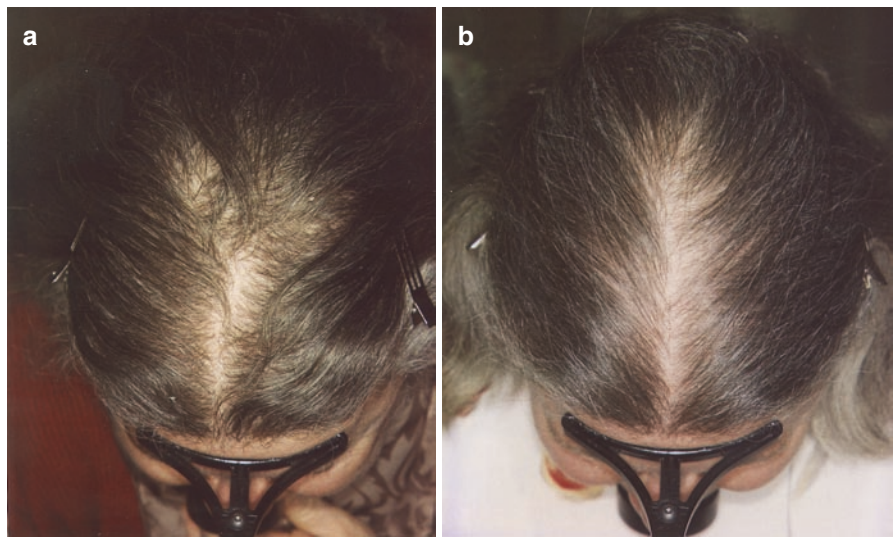

Fig. 15.9 Supplementation with cystine and vitamin B-complex: (**a**) before and, (**b**) after 6 months of oral therapy (from Lengg et al. [43])

levels above the lower normal limit of 10 μg/L did not influence changes in anagen hair rate either, this again in agreement with others who have challenged the recommendation of iron supplementation for women with hair loss and serum ferritin levels within the normal limits [4, 11, 75].

15.3.12 Aging and Diet

At present, the only scientifically documented means of slowing aging through dietary changes is by caloric restriction. It must be kept in mind though, that an anti-aging diet is significantly different than a weight loss diet. The key efforts of an anti-aging diet focus more importantly on altering body composition to keep the organism at near-peak performance during the advancing years. The approach covers a decrease of calories intake by approximately 30% daily, addition of high-density nutrients, and improvement of digestion and gastrointestinal function. Moreover, there is evidence that with age, the needs for types and quantities of nutrients may change. Since an important commercial interest lies in the nutritional value of various vitamin and amino acid supplements, finally, another question that arises is whether increasing the content of an already adequate diet with specific amino acids, vitamins, and trace elements may further promote hair growth in the elderly.

15.4 Endocrine Disease in the Elderly

Anti-aging medicine is concerned with discovering and applying therapies to modify, alleviate, or slow the progressive afflictions associated with age. Besides nutrition, vitamins, and supplements, the anti-aging marketplaces focus on hormone replacement therapies under the assumption that a decline of hormone production with age is closely related to the aging process. In both sexes, growth hormones, melatonin, dehydroepiandrosterone (DHEA), and its sulfate compound DHEAS reach their maximum levels in the third decade of life, and then decline progressively. In addition, a constant decrease in the production of biologically active free testosterone of approximately 1% per year is observed in men. The abrupt cessation of sex hormone production seen in women is not observed in men.

Millions of women have used synthetic estrogens to reduce the impact of menopause. Starting in 1991, the Women's Health Initiative studied 161,808 postmenopausal women with randomized trials of hormone supplements vs. placebo. The study was discontinued in 2002, because it concluded that on the whole the supplements were doing more harm than good [3, 65]. Millions of men are taking testosterone supplements, as creams or patches, and tens of thousands of Americans are taking human GH injections. DHEA, pregnenolone, melatonin, and thyroid are yet other hormones considered as anti-aging agents. Despite some promising results in a variety of studies conducted over the past years, presently available data do not justify the broad use of hormones for anti-aging purposes. More important is to recognize pathologic conditions and treat them appropriately.

Hormones relevant for the condition of the hair are: androgens, estrogens, thyroid and parathyroid hormones, prolactin, corticosteroids, GH/IGF-1, and melatonin.

15.4.1 Effect of Menopause on Hair

Menopause is based on the cessation of hormone production by the ovaries, in this case, of the hormones that make reproduction possible and may influence sexual behavior. The resultant decreased levels of circulating estrogen impacts the entire cascade of a woman's reproductive functioning, from brain to skin. The typical age range for the occurrence of menopause is between the age of 45 and 55. The average age of menopause varies according to geographic location. In the Western world, the average age of menopause is 51 years, in some developing countries, such as India and the Philippines, the median age of natural menopause is earlier, at 44 years [64]. Dermatologic problems that postmenopausal women encounter include atrophy, dryness, pruritus, loss of resilience and pliability, easily traumatized skin, dry hair, and alopecia [60]. Those effects are currently understood to be due to low estrogen levels.

Estrogens clearly have an important function in many components of human skin including the epidermis, dermis, vasculature, hair follicle, and sebaceous, eccrine, and apocrine glands, having significant roles in skin aging, pigmentation, hair growth, and sebum production [77]. Although androgens have dominated endocrinologic research in hair growth control, and androgen

metabolism and the androgen receptor have been the key targets for systemic, pharmacological treatment of androgenetic alopecia in dermatological practice, it has long been known that estrogens influence hair follicle growth and cycling by binding to locally expressed high-affinity estrogen receptors (ERs). The discovery of a second intracellular estrogen receptor (ERbeta) with different cell-specific roles to the classic estrogen receptor (ERalpha), and the identification of cell surface estrogen receptors in the hair follicle, has provided further challenges to understand the mechanism of estrogen action on hair growth [53]. Besides altering the transcription of genes with estrogen-responsive elements, 17beta-estradiol (E2) also modifies androgen metabolism within the pilosebaceous unit, which itself displays prominent aromatase activity, the key enzyme for androgen conversion to E2. Therefore, the hair follicle is both a target and source for estrogen [54].

Clinical evidences for the role of estrogens for hair growth are observations of the effects of pregnancy, hormonal treatments that affect estrogen metabolism, and menopause on the condition of the hair. During the second half of pregnancy, the percentage of anagen hairs increase from the normal 85% to 95% [47], at this time also the percentage of hairs of large shaft diameter is higher than in nonpregnant women of the same age [58]. After partuition, the follicles, in which anagen has been prolonged, rapidly enter catagen and then telogen, with an increased shedding of hair evident after 1–4 months (postpartum effluvium). The observation that many women show increased shedding of hair from 2 weeks to 3–4 months after they stop taking an oral contraceptive probably simulates that, which is commonly seen after parturition [21]. More frequently, contraceptive pills or hormone replacement therapies with progestogens that possess net androgenic activity (norethisterone, levonorgestrel, tibolone) induce common baldness in genetically predisposed women. It has been proposed that in the presence of a genetic susceptibility, it is the estrogen to androgen ratio that might be responsible for triggering hair loss in women [63]. In the same line is the observation of hair loss induced in the susceptible women by treatment with aromatase inhibitors for breast cancer [16]. Finally, postmenopausal women show an increased tendency toward male pattern hair loss (Fig. 15.10) [82].

With respect to menopause and hormonal substitution therapy, the focus tends to be on the issues recently covered by the Women's Health Initiative [65]. Consequently, many women have become reluctant toward systemic estrogen substitution therapy. Topical estrogen supplementation with E2 or its stereoisomer 17alpha-estradiol (alfatradiol) has been suggested to be of some benefit [1, 8, 32, 55, 56]. Unfortunately, these studies are relatively small, and the results for alfatradiol are not convincing [8]. Also no literature exists comparing the efficacy and safety of topical versus systemic estrogens.

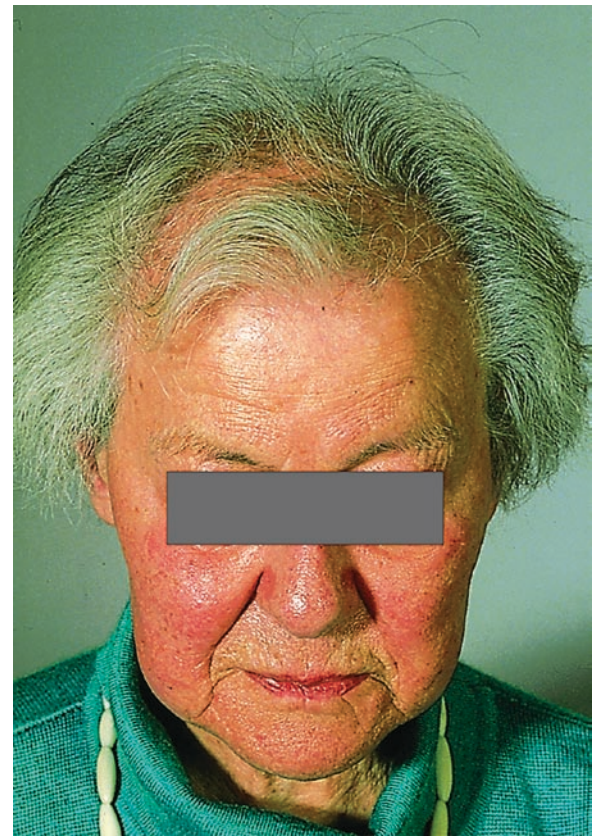

Fig. 15.10 Male pattern baldness in postmenopausal woman

15.4.2 Thyroid Disorders

Thyroid hormones have influence on the growth and differentiation of many tissues and total energy expenditure of the organism, and on the turnover of many substrates, vitamins, and other hormones. Thyroid activity affects oxygen consumption, protein

synthesis, and mitosis, and is therefore essential for the formation and growth of hair. Expression of the thyroid hormone receptor beta1 was demonstrated in the human hair follicle, and it was shown that trijodo-L-thyronine significantly enhances human hair survival in vitro [7]. The impact of thyroid hormone activity on the hair is most notable during deficient or excess states. Schell et al. [73] demonstrated for the first time by means of DNA flow cytometry the influence of thyroid hormones on in vivo cell cycle kinetics of human scalp hair bulbs. Clinically, the effect of thyroid disorders on hair is nonspecific, nevertheless the associated symptoms and signs of thyroid hormone deficiency or excess may provide an important clue to the diagnosis in instances of unsuspected thyroid disease.

Hypothyroidism results from a deficiency of thyroid hormones. It is caused most commonly by chronic autoimmune thyroiditis (Hashimoto's disease) or by iatrogenic thyroid ablation (I^{131} therapy or surgical thyroidectomy). Iodine deficiency is rare in developed countries, but is common in some regions of the world. Hypothyroidism is about ten times more frequent in women than in men, and particularly affects women between the ages of 40 and 60 years. Patients present with dry, rough skin, and in severe cases, this can simulate ichthyosis. The facial skin has puffy features with increased skin creases, and patients may have a flat, expressionless face. Dull, coarse, brittle hair or diffuse alopecia may be present with particular thinning of the lateral eyebrows. The hair growth rate is slowed, with an increase in the proportion in telogen. The alopecia is of very gradual onset. Longstanding hypothyroidism may be associated, in the genetically predisposed, with androgenetic alopecia (Fig. 15.11) [39]. The mechanism is presumed to be due to an increase in plasma free androgens. Such symptoms may easily be overlooked or ascribed merely to aging.

Hyperthyroidism is due to excessive quantities of circulating thyroid hormones. Graves' disease is by far the most common cause with an estimated prevalence of 5.9% in a population of patients 60 years and older [72]. Again, it is a disease of autoimmune origin affecting women much more frequently than men. The most common symptoms of hyperthyroidism are systemic rather than cutaneous and due to a hypermetabolic state known as thyrotoxicosis. Nevertheless, diffuse hair loss is present in 20–40% and axillary hair loss in 60% (Fig. 15.12) [87]. The severity of alopecia does not correlate with the severity of thyrotoxicosis. The hair itself is fine, soft, straight, and allegedly unable to retain a permanent wave.

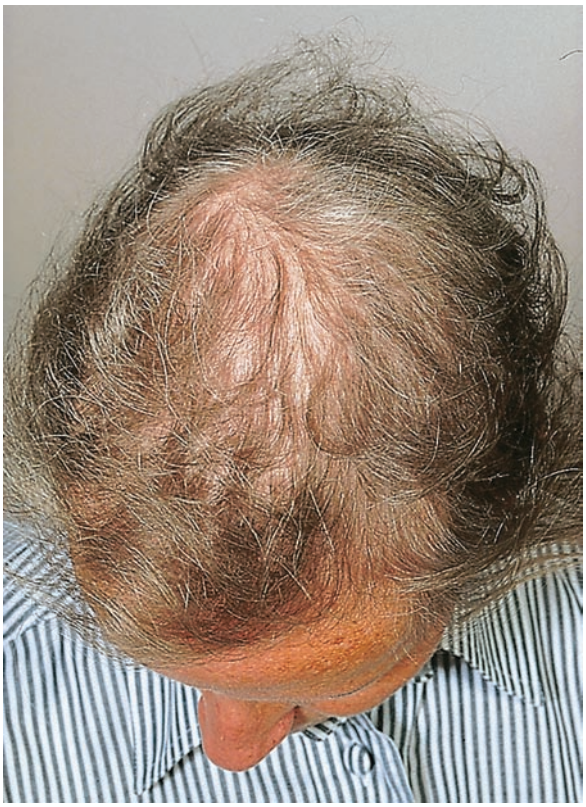

Fig. 15.11 Hypothyroidism: alopecia

It must be kept in mind that also medications for treatment of thyroid disease or medications that interfere with thyroid metabolism may be the cause of hair loss: carbimazol, thiamazol, methylthiouracil, propylthiouracil, iodine, levothyroxine, lithium, and amiodarone.

Hypoparathyroidism is seen most commonly in the geriatric population following inadvertent removal of the parathyroid glands during thyroid surgery or radical neck dissection for cancer. Patients suffer attacks of hypocalcemia with tenany. Thinning of the hair or complete alopecia may be seen. The nails commonly develop horizontal grooves on the nail (Beau's lines) that appear at the base of the nails approximately 3 weeks following a titanic attack. Enamel defects of the teeth may be misinterpreted as from poor oral hygiene especially in the elderly. Chronic disease manifestations as visual blurring from cataracts, Parkinson's disease, lethargy, and personality changes may again falsely be attributed to aging by the patient and physician.

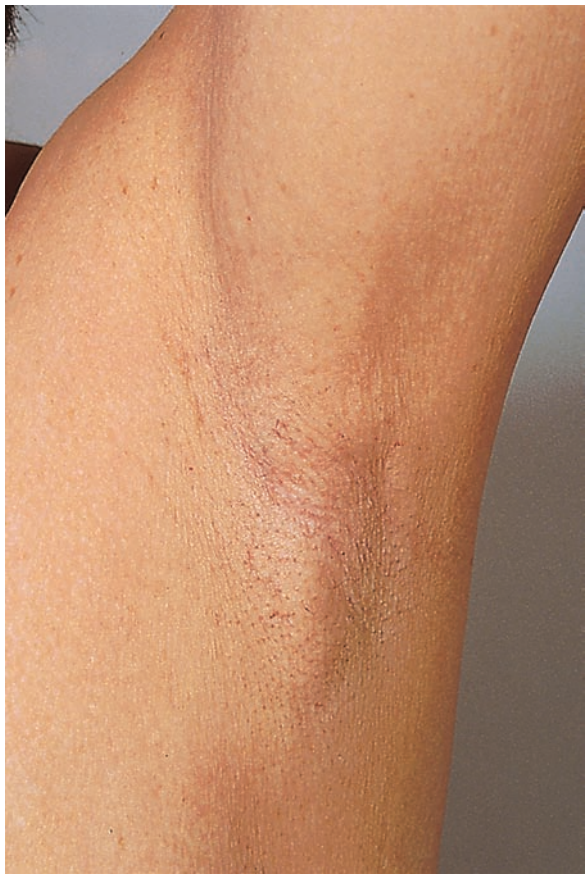

Fig. 15.12 Hyperthyroidism: axillary hair loss

15.4.3 Adrenal Disorders

Hormones of the steroid family (glucocorticoids, androgens, and mineralocorticoids) control cell growth, differentiation, and metabolism. Adrenal disorders may result in excessive glucocorticoid activity (hypercortisolism or Cushing's syndrome), insufficient glucocorticoid activity (adrenal insufficiency or Addison's disease), excessive androgen activity, or deficient androgen activity.

Hypercortisolism or Cushing's syndrome refers to the manifestations of increased cortisol secretion by the adrenal glands for any reason. It is most commonly the result of iatrogenic administration of glucocorticosteroids, but the same findings are present in patients with endogeneous hypercortisolism, from pituitary adrenocorticotropic hormone (ACTH) production (Cushing's disease), adrenal tumors, or ectopic ACTH production. Hypertension and weight gain are early manifestations; the typical cutaneous manifestations include redistribution of body fat, with central obesity, "buffalo hump" "moon face" and thin arms, and atrophic skin, with easy bruisability, pigmented facial hypertrichosis, general increase in lanugo hair (Fig. 15.13), and alopecia. These effects may be dismissed initially as being secondary to normal aging of the skin.

Addison's disease is a chronic adrenocortical insufficiency. Autoimmune destruction of the adrenals accounts for the majority of cases, with metastasis to the adrenals being another potential cause and tuberculosis in regions where the infection is still prevalent. The most striking dermatologic sign is increased skin pigmentation; the hair may also become darker.

Excessive androgen activity is reflected in precocious puberty in children, and virilization in women, whereas men are asymptomatic. Androgen excess may be caused by a wide variety of conditions of both adrenal and ovarian origin. These include: congenital adrenal hyperplasia or adrenogenital syndromes, adrenal tumors, Cushing's syndrome, polycystic ovaries, ovarian tumors, and other nonadrenal, nonovarian neoplasms. The cutaneous signs of virilization include hirsutism (Fig. 15.14) and androgenetic alopecia. Abrupt onset of signs of virilization, DHEAS level greater than 600 ng/L, and free testosterone levels greater than 200 ng/L should suggest an androgen-producing tumor. The *adrenogenital syndromes* result from genetically determined defects in the synthesis of cortisol. An increase in ACTH production resulting in increased adrenal stimulation combined with the blockade in the cortisol production pathway leads to an accumulation of adrenal androgens. This causes

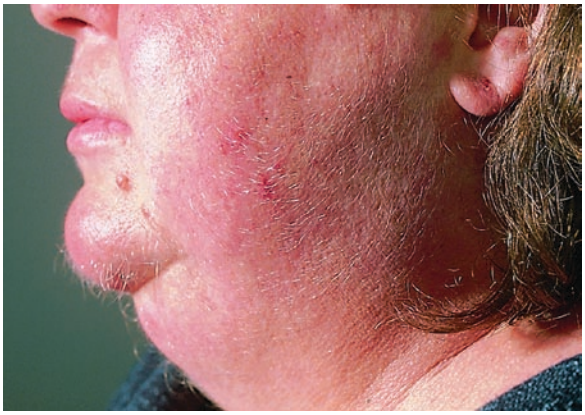

Fig. 15.13 Cushing's syndrome: "moon face" with hypertrichosis

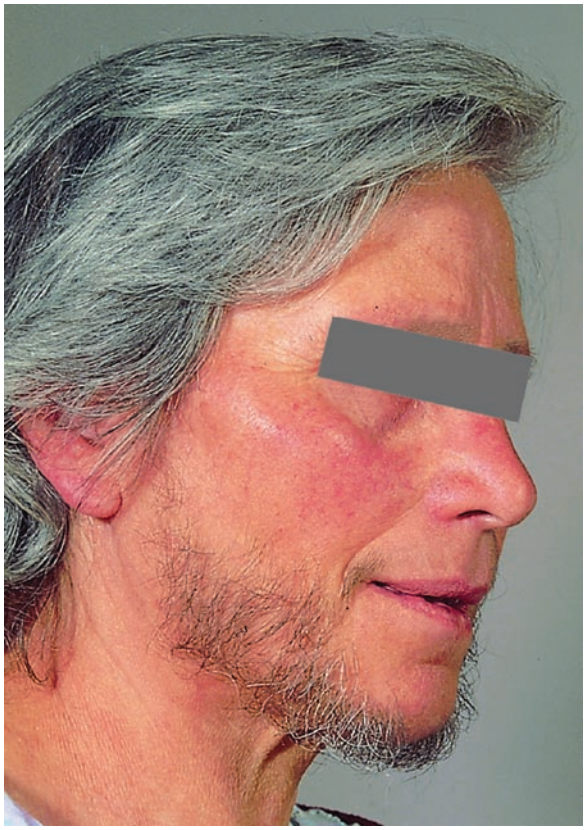

Fig. 15.14 Virilization from androgen-producing tumor: facial hirsutism

virilization in women. *Partial 21-hydroxylase deficiency* can present with hirsutism, even in older women.

Deficient androgen activity may result in the decrease of sexual desire and arousability, loss of muscle tone, diminished vital energy, and dry skin. With the development of androgen deficiency after puberty, sexual hair is present but grows slowly, since maintenance of sexual hair is less dependent on androgen than is the development of sexual hair.

15.4.4 Pituitary Disorders

Hypopituitarism may manifest itself as an isolated deficiency of one or many anterior pituitary hormones, resulting in secondary atrophy of the gonads, thyroid, and adrenal cortex.

Panhypopituitarism (*Simmonds' disease*) is the total absence of all known pituitary hormones. The most common causes of hypopituitarism in the adult are infarcts (postpartum necrosis of Sheehan) or ablation of the gland by surgery. The clinical manifestations of hypopituitarism vary with the type, age of development, and degree of hormonal deficiencies. The most common cutaneous manifestations are pallor with decreased ability to tan, the texture of the skin is smooth, fine wrinkling of the face and forehead is said to be characteristic, and the body hair is scant.

GH deficiency is the medical condition of inadequate production of GH. Deficiency of GH produces significantly different problems at various ages. In newborn infants, the primary manifestations may be hypoglycemia or micropenis. In later infancy and childhood, growth failure may be the major effect. Though growth ends with sexual maturity, GH continues to be secreted throughout life. In adults, GH contributes to maintenance of muscle and bone mass and strength as well as quality and productivity in life. GH deficiency in adults is rare, its causes include: damage to the pituitary from intracranial disease (e.g., hydrocephalus), intracranial tumors in or near the sella turcica, especially craniopharyngeoma, damage to the pituitary from radiation therapy to the head for leukemia or brain tumors, surgery in the area of the pituitary, autoimmune inflammation (hypophysitis), severe head trauma, ischemic or hemorrhagic infarction from low blood pressure (Sheehan syndrome), or hemorrhage pituitary apoplexy. GH secretion declines progressively with aging, and many age-related changes resemble those of the adult GH deficiency syndrome, including decrease in lean body mass, increase in body fat, especially in the visceral/abdominal compartment, adverse changes in lipoproteins, and a reduction in aerobic capacity. GH replacement is effective in reversing many of these changes in adult GH deficiency, and GH is FDA approved for the treatment of adults with documented GH deficiency or hypopituitarism. This experience with GH deficiency has led to the speculation that replacing GH may also be beneficial in normal aging, and to widespread off-label use of GH in this context. However, its safety, efficacy and role in healthy elderly individuals is highly controversial.

Laron syndrome is an autosomal recessive disorder characterized by insensitivity to GH, caused by a variant of the GH receptor. Molecular genetic investigations have shown that this disorder is mainly associated with mutations in the gene for the GH receptor. These can result in defective hormone binding to the ectodomain or reduced efficiency of dimerization of the receptor

after hormone occupancy. There are exceptionally low levels of IGF-1 and its principal carrier protein, insulin-like growth factor binding protein 3 (IGFBP3). Administration of GH has no effect on IGF-1 production; therefore, treatment is mainly by biosynthetic IGF-1. It causes short stature. Physical symptoms include: prominent forehead, depressed nasal bridge, underdevelopment of mandible, truncal obesity, and a very small penis. Hair manifestations are hypotrichosis, premature alopecia, and unspecific hair shaft anomalies [44].

Hyperpituitarism is generally secondary to a pituitary tumor resulting in the secretion of one specific hormone.

Acromegaly results from hypersecretion of GH. It occurs more commonly in adults and leads to exaggerated growth of acral parts, particularly the head, hands, and feet, the skin is thickened and doughy, the skin pores are usually prominent, the skin of the face is oily, and furrows and ridges are seen about the face, neck, and scalp and are referred to as *cutis verticis gyrata*. Approximately 50% of patients have increased body and scalp hair, and the hair is coarse.

Hyperprolactinemia is a condition of elevated serum prolactin. Prolactin is produced in the anterior pituitary gland. Its primary function is to enhance breast development during pregnancy and to induce lactation. Elevations in prolactin levels are seen in pituitary hypersecretion, in association with the use of psychotropic drugs and prolactinomas. Data suggest that hyperprolactinemia may be responsible for abnormal androgen production [17], resulting in hirsutism and alopecia [55].

15.4.5 Hormonal Treatments for Anti-Aging

In the course of hormonal anti-aging protocols containing recombinant human GH, Edmund Chein from the Palm Springs Life Extension Institute reports improvement of hair thickness and structure in 38% of patients, in some cases darkening of hair, and in few increased hair growth [20].

Hormone therapy with androgens, androgen precursors (DHEA), or progestins with androgenic action (norethisterone, levonorgestrel, tibolone) may cause hair loss in individuals with androgenetic alopecia.

Melatonin, the chief secretory product of the pineal gland, is known to modulate hair growth and pigmentation, presumably as a key neuroendocrine regulator that couples coat phenotype and function to photoperiod-dependent environmental and reproductive changes. Important extrapineal melatonin synthesis has recently been demonstrated in human scalp hair follicles in anagen, where melatonin may also functionally play a role in hair-cycle control, as it downregulates apoptosis [27]. To examine whether topically applied melatonin influences hair growth and shedding in otherwise healthy women complaining of hair loss, a double-blind, randomized, placebo-controlled study was conducted in 40 women. A 0.1% melatonin or a placebo solution was applied on the scalp once daily for 6 months and trichograms were performed. This pilot study was the first to show that topical melatonin influences hair growth in humans in vivo. The mode of action was hypothesized to result from an induction of anagen phase [26]. Because of melatonin's additional property as a free radical scavenger and DNA repair inducer, the metabolically and proliferatively highly active anagen hair bulb may also exploit melatonin synthesis *in loco* as a self-cytoprotective strategy [27].

15.5 Psychological Disorders in the Elderly

Many patients with a hair or scalp disorder have psychological issues associated with their chief complaint. Because so many different types of conditions lie in between the fields of dermatology and psychiatry, it is paramount to have classification systems that will help the clinician understand what he is dealing with. There are two ways to classify psychocutaneous cases: first, by the category of the dermatologic presentation and second, by the nature of the underlying psychopathologic condition. Since the clinical presentations are quite stereotypic, but the underlying psychopathology is various, a critical step in psychodermatology is to try to ascertain the nature of the underlying psychopathologic condition.

15.5.1 Generalized Anxiety Disorder

Generalized anxiety disorder is characterized by a sustained, increased free floating anxiety, which is

not orientated toward a certain object or situation. It expresses itself in the form of anxious expectations and enhanced alertness, combined with motorical hypertension and, as a physiological correlate, vegetative hyperreactivity. Subjective symptoms include feelings of restlessness, irritability, feeling "on edge" tension, dizziness, agitation, and an inability to relax. These are frequently associated with physiological correlates such as muscle tension, sweating, shortness of breath, dry mouth, palpitations, abdominal complaints, and frequent urination. When patients with psychophysiological disorders complain that they are "stressed" they are usually referring to an underlying sense of anxiety. The uninhibited breakthroughs of tremendous anxiety show that the anxiety defense mechanisms failed in the affected individuals. The causes of anxiety are repressed, but the ongoing arousal and fear are overwhelming. The patients' appearance is clinging and helpless. The patients signify a strong demand to be guided and assisted to their surroundings.

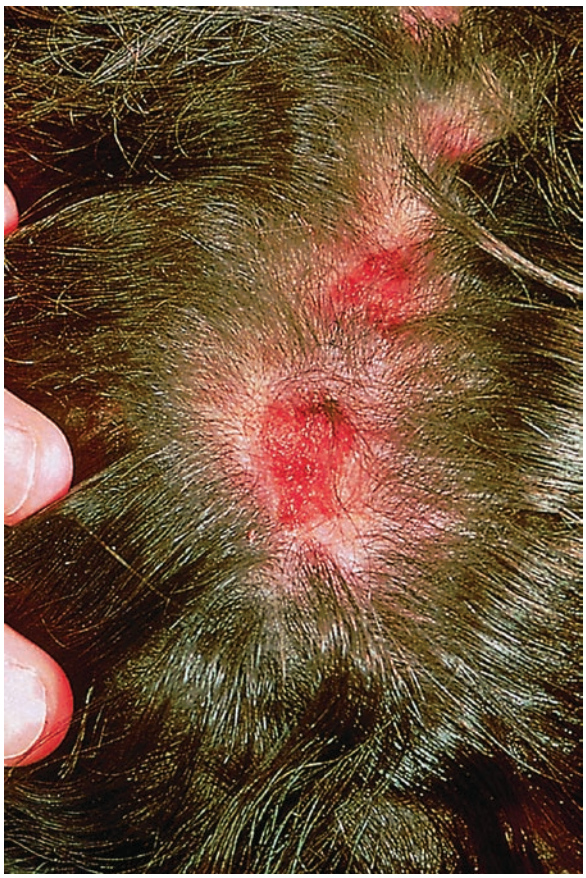

Fig. 15.15 Generalized anxiety disorder: neurotic excoriations of scalp

Manifestations of generalized anxiety disorder on the scalp may be scalp dysesthesia, or neurotic excoriations of the scalp (Fig. 15.15).

15.5.2 Depressive Disorder

In a depressive disorder, the affected individual suffers from the symptoms of a depressive syndrome, which may be intermitted by shorter or longer periods of normal mood. Depression is characterized by subjective symptoms, such as depressed mood, crying spells, anhedonia (inability to experience pleasure), sense of helplessness, hopelessness and worthlessness, excessive guilt, and suicidal ideation. Frequently associated physiological correlates are psychomotor retardation or agitation, insomnia or hypersomnia, loss of appetite or hyperphagia, and, especially in older patients, complaints of constipation.

Manifestations of depressive disorder on the scalp may be scalp dysesthesia, neurotic excoriations of the scalp, or psychogenic pseudoeffluvium (Fig. 15.16).

Psychogenic pseudoeffluvium is the term coined for the fear of the possibility or the conviction of going bald without any objective findings of hair loss.

15.5.3 Adjustment Disorders

An adjustment disorder is a debilitating reaction to a stressful event or situation. Some patients have difficulties adjusting to hair loss. As a result, the individual may have difficulty with his or her mood and behavior. The symptoms or behaviors are clinically significant as evidenced by either of the following: distress that is in excess of what would be expected, significant impairment in social, occupational, or educational functioning. Adjustment disorders subtypes are: adjustment disorder with depressed mood, adjustment disorder with anxiety, adjustment disorder with mixed anxiety and depressed mood, adjustment disorder with disturbance of conduct, and adjustment disorder with mixed disturbance of emotions and conduct. Associated features may be somatic or sexual dysfunction and feelings of guilt or obsession.

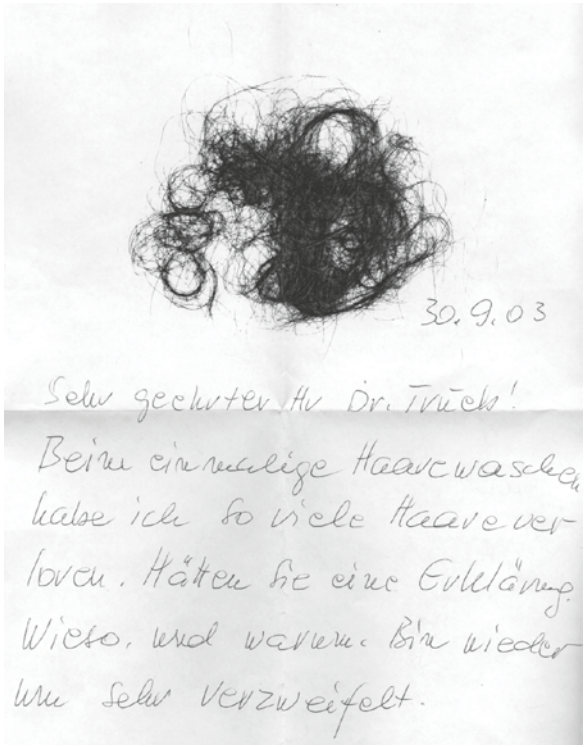

Fig. 15.16 Depressive disorder: letter from patient with psychogenic pseudoeffluvium

Probably the best way to alleviate the emotional distress caused by hair disease is to eliminate the hair disease that is causing the problem. In other words, the intensity of the distress that the patient feels should be part of the clinician's formula in deciding how aggressively to treat the hair disease.

15.5.4 Delusional Disorder

Presence of delusion defines psychosis. A delusion is a false idea that the patient is absolutely fixed on. By definition, a delusional patient has no insight, and others cannot talk him out of his belief system. A delusion is deemed as a basic psychotic phenomenon, in which the objective falseness and impossibility of the delusional content is usually easy to realize. Delusional convictions are not simple misbeliefs; they are constitutions of an own, abnormal mind that refers to the individual's cognitive experiences with the environment and the ego-environmental relationship. Delusions are not voluntarily invented by the patients; they were caused by psychotic experiences. From the psychodynamic point of view, a delusional disorder is a special consequence of an abnormal self-development. The delusion's necessity comes off the psychotic tension, caused by the brittleness and contradictoriness of the patient's environmental- and ego-experiences, where he is now trying to reach, with the help of the delusions, safety. The subjective certainty of the delusion's content causes its incorrectability: the patient consistently keeps his convictions, without considering the incompatibleness with reality. Neither contrary experiences, nor logical arguing can influence him.

Manifestations of delusional disorder on the scalp may be psychogenic pseudoeffluvium, delusion of parasitosis, or trichophobia.

In *delusion of parasitosis*, there is an unshakable conviction that the skin is infested by parasites [46, 89]. In the medical literature, the typical patient is reported to be a middle-aged woman. Though the patient with delusions of parasitosis presenting to the dermatologist more frequently suffers from monosymptomatic hypochondriacal psychosis, it must be kept in mind that the presence of a delusional ideation may be one particular manifestation of a more global psychiatric derangement, such as schizophrenia or major depression. Another subset of patients to consider are those patients who are substance abusers: Drugs such as cocaine and amphetamine can induce formication and, sometimes, a delusional state that can be clinically identical to that of idiopathic delusions of parasitosis. Also, neurological disorders such as multiple sclerosis [57] and pernicious anemia [61], and especially in the elderly, brain dysfunction with manifest encephalomalacia due to cerebral arteriosclerosis [49], are to be considered in the differential diagnosis.

Trichophobia denotes plucking of hair on the basis of the delusion of having to pull something out of the hair roots [42].

15.5.5 Cutaneous Sensory Disorders

Some patients only present with a cutaneous sensory complaint such as itching, burning, stinging, or other disagreeable sensations of the scalp without any diagnosable dermatologic, neurologic, or medical diagnosis. Patients with chronic cutaneous sensory disturbance of unknown etiology can be divided into those with

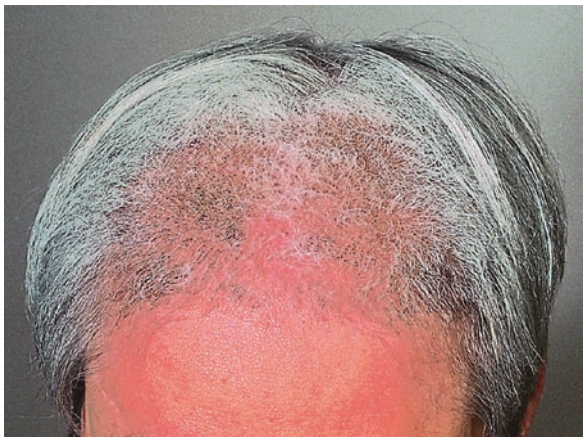

Fig. 15.17 Cutaneous sensory disorder: trichoteiromania

diagnosable psychiatric findings, such as a depression or anxiety, and those with no diagnosable psychiatric findings. The latter patients suffer from *somatoform pain disorder*. Albeit in dermatology the somatoform disorders consist of a heterogeneous pattern of differing clinical presentations, they are based on a comparable emotional disorder, the characteristic of which is repeated presentation of physical symptoms in combination with a stubborn demand for medical examination, despite repeated negative results and the physician's assurance that the symptoms have no physical basis.

Trichoteiromania is the term coined for breakage of hair in the area of forceful rubbing of the scalp associated with a sensory disorder of the scalp (Fig. 15.17) [29].

15.5.6 Factitious Disorderssss

Factitious disorder is a condition in which a person deliberately produces, feigns, or exaggerates symptoms to satisfy a psychological need of which he or she is not consciously aware, usually a need to be taken care of by assuming the sick role [25, 30, 37, 45, 76]. Patients with factitious disorder or factitial dermatitis do so for psychological reasons, and not for monetary or other discrete objectives as in the case of malingering. Patients knowingly fake symptoms, but will deny any part in the process. They desire the sick role and may move from physician to physician to receive care.

Little is known about the etiology of factitious disorder. Besides the difficulties involving the diagnosis, reluctance of those patients to undergo psychological testing and heterogeneity in details of cases published in literature are at the origin of this situation. A majority of patients suffer from the borderline personality disorder. In all reported series, female outnumber male patients from 3:1 to 20:1, and there is a remarkably high number of patients who work, or who have a close family member working, in the health care field.

Factitial dermatitis of the scalp is only one aspect of the whole picture of factitious disease. The condition for which dermatologists are consulted often has already occasioned many visits to other physicians. The patient typically presents a bundle of normal investigative findings and a shopping bag filled with oral and topical medications. The lesions themselves are as varied as the different methods employed to create them; usually ulcerations (Fig. 15.18) or areas of cut off hair are seen on the scalp. They are bizarre in shape and distribution, and usually appear on normal

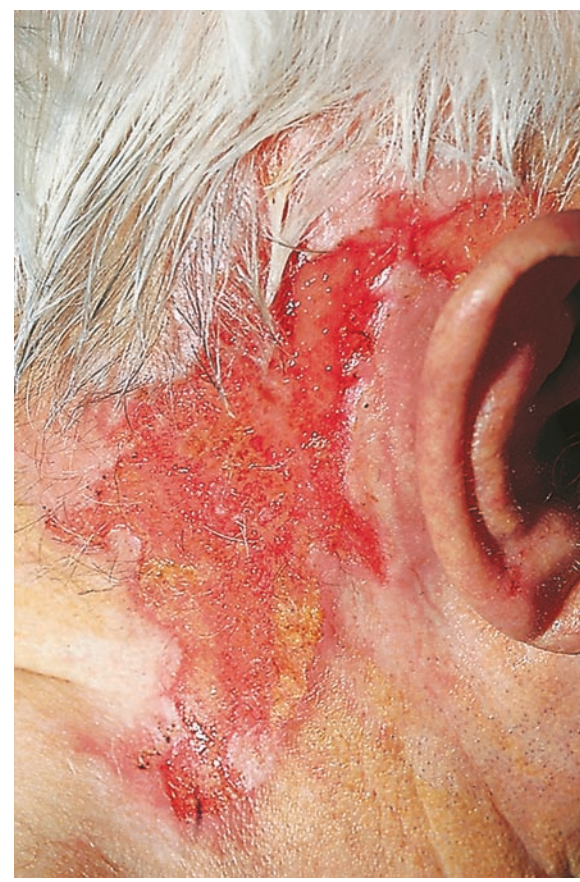

Fig. 15.18 Factitious disorder: traumatogenic scalp ulceration

skin. Though the possibilities are limitless, consistent is a "hollow" history, a term that refers to a vagueness and inability of the patient to give details of the evolution of the lesions. Also consistent is the affect of the patient and family. Although the patient seems astonishingly unmoved by the lesions, the family is angry, accusatory, and critical of what they interpret as medical incompetence.

Trichotemnomania is the term coined for self-inflicted hair loss through deliberate cutting of hair [10].

15.5.7 Obsessive-Compulsive Disorder

An obsession is a persistent idea, thought, impulse, or image that intrudes into a person's consciousness uncontrollably and causes distress, anxiety, and often feelings of shame. The obsessional concerns often lead to compulsive acts. Compulsions are repetitive, stereotyped motor acts, often ritualized, and designed to reduce intolerable anxiety or distress. The essential feature of obsessive-compulsive disorder required for diagnosis is recurrent obsessions or compulsions that are severe enough to be time-consuming or cause impairment in relationships, employment, or social activities. By definition, patients suffering from obsessive-compulsive disorder have insight into their condition, in contrast to delusional patients. The individual with obsessive-compulsive disorder realizes that the obsession is inappropriate and irrational, but cannot resist. Obsessions may involve themes of aggression (harming the self or others), contamination (dirt, germs, body secretions), sex (forbidden thoughts or impulses), religion (concern with blasphemy or sacrilege), or somatic concerns.

Manifestations of obsessive-compulsive disorder on the scalp may be neurotic excoriations of the scalp or trichotillomania.

Trichotillomania is the term coined for the repeated urge to pull out scalp hair, eyelashes, facial hair, nose hair, pubic hair, eyebrows, or other body hair, sometimes resulting in noticeable bald patches. Trichotillomania is diagnosed in all age groups, though it is substantially more common during the first two decades of life. Whereas among preschool children the genders are equally represented, there is a female predominance among adults. In the elderly, trichophobia (a delusional disorder), trichoteiromania (secondary to cutaneous sensory disorders), or trichotemnomania (a factitious disorder) are more frequently seen than trichotillomania.

15.5.8 Body Dysmorphic Disorder

Patients with body dysmorphic disorder become preoccupied with a nonexistent or minimal cosmetic defect and persistently seek medical attention to correct it. The patient is preoccupied with an imagined defect of appearance or is excessively concerned about a slight physical anomaly. This preoccupation causes clinically important distress or impairs work and social or personal functioning. One of the various theories attempting to make the onset of body dysmorphic disorder understandable is the "self-discrepancy theory", in which affected patients present conflicting self-beliefs with discrepancies between their self-being and wishing to be. Patients have an unrealistic ideal as to how they should look. Media-induced factors are considered to predispose to body dysmorphic disorder by establishing role models for beauty and attractivity.

The more recently described *Dorian Gray syndrome* [12] probably represents a variant of body dysmorphic disorder, in which patients wish to remain for ever young and seek lifestyle drugs and surgery to deter the natural aging process. It has been named after a novel of Oscar Wilde, in which the protagonist, a beautiful young esthete, exclaims in front of his portrait: "Why should it keep what I must lose? Every moment that passes takes something from me, and gives something to it. Oh, if it were only the other way! If the picture could change, and I could be always what I am now!" [86].

15.5.9 Dementia

Dementia (from: "de-"= apart, away + "mens" (genitive: "mentis") = mind) is the progressive decline in cognitive function due to damage or disease in the body beyond what might be expected from normal aging. It represents a nonspecific set of symptoms, in which affected areas of cognition may be memory, attention, language, and problem solving. Higher mental functions are affected first in the process, and in the later stages of the condition, affected persons may be disoriented in time, in place, and in person.

Some cases of dementia are due to different specific disease processes that may be reversed with treatment. Out of this reason, routine blood tests are recommended to rule out treatable causes. These include: vitamin B12, folic acid, thyroid-stimulating hormone (TSH), C-reactive protein (CRP), full blood count, electrolytes, calcium, renal function, liver enzymes, and syphilis serology. Abnormalities may suggest vitamin deficiency, infection, or other problems that commonly cause confusion or disorientation in the elderly. The problem is complicated by the fact that these cause confusion more often in persons who have early dementia, so that "reversal" of such problems may ultimately be only temporary.

Also mental illnesses, including depression and psychosis, may produce symptoms that must be differentiated from dementia (pseudo-dementia). Chronic abuse of substances, such as alcohol, can also predispose to cognitive changes suggestive of dementia.

Manifestations of dementia on the scalp are neglecting hair and hair care.

15.6 Drug-Related Problems in the Elderly

In general, the incidence of drug-related adverse events increases with age owing to a higher susceptibility to drug-related side-effects, a higher frequency of medications, comedications, and comorbidities in older patients. Adverse drug reactions on the hair are common. Drugs are capable of producing a wide spectrum of hair loss, ranging from barely detectable shedding to frank and irreversible baldness. Other side-effects observed are the stimulation of hair growth with hypertrichosis or induction or worsening of hirsutism, as well as changes in hair structure or color.

15.6.1 Drug-Induced Alopecia

Drug-induced hair loss is usually diffuse nonscarring alopecia, which is reversible upon withdrawal of the drug. Only a few drugs, mainly antimitotic agents, regularly cause hair loss, whereas many drugs may be the cause of isolated cases of alopecia. There is a long list of drugs that on occasion have been cited as causing hair loss (Table 15.1): all anticoagulant and antithyroid drugs can produce hair loss; some psychotropic drugs are very likely to induce a drug-related alopecia; it has been reported that some patients taking lithium developed hair thinning; case reports with tricyclic antidepressants rarely appear in the literature; hair loss is reported secondary to some anticonvulsant agents, mainly valproic acid, among antihypertensive drugs; ACE inhibitors, systemic or topic beta-adrenoceptor antagonists (for treatment of glaucoma) should be considered as possible causes of hair loss; hair loss from nonsteroidal analgesics occurs in a very small percentage of patients; a few isolated cases have been reported with some hypocholesterolemic or anti-infectious agents. Diagnosis of drug-induced alopecia remains a challenge. The only way to confirm it is to see if an improvement occurs after cessation of the suspected drug. This side-effect must be recognized, because it may be a source of poor compliance in some patients.

15.6.2 Chemotherapy-Induced Alopecia

Few dermatologic conditions carry as much emotional distress as chemotherapy-induced alopecia. It is considered as one of the most negative factors in cancer patient care, and occurs with an estimated incidence of 65%. Hair loss negatively affects a patient's perception of appearance, body image, sexuality, and self-esteem. Females are particularly affected. Moreover, patients feel deprived of their privacy of having cancer. A survey demonstrates that 47% of female cancer patients consider chemotherapy-induced alopecia as the most traumatic aspect of chemotherapy, and 8% would even decline chemotherapy owing to fear of hair loss [50, 52].

The three major and most frequent toxicities of cytotoxic cancer therapy are bone marrow suppressions, gastrointestinal disturbances, and alopecia. This type of injury is the consequence of a direct toxic insult to the rapidly dividing cells of the bone marrow, gastrointestinal tract, and hair follicle, respectively. Clearly, chemotherapy-induced alopecia differs from the more common type of telogen effluvium, also in terms of clinical presentation. It is a major characteristic of the anagen hair follicle that the epithelial compartment undergoes proliferation, with the bulb matrix

Table 15.1 Drug-induced hair loss (from Trüeb [81])

Dystrophic anagen effluvium due to inhibition of mitosis: cytostatic agents	
Cytostatic agents that usually do cause hair loss	Adriamycin Docetaxel Daunorubicin Paclitaxel Etoposide Ifosfamide Irinotecan Vindesine Cyclophosphamide Vinorelbine Epirubicin Topotecan
Cytotoxic agents that sometimes cause hair loss	Amsacrine Vincristine Cytarabine Vinblastine Bleomycin Lomustine Busulfan Thiotepa 5-Fluorouracil Gemcitabine Melphalan
Cytotoxic agents that unusually cause hair loss	Methotrexate Procarbazine Carmustine 6-Marcaptopurine Mitroxantrone Streptozotocin Mitomycin C Fludarabine Carboplatin Raltritrexed Cisplatin Capecitabine
Telogen effluvium with known or assumed mechanism	
Interference with keratinization process in the hair follicle: retinoids	Vitamin A (>50,000 I.E. daily) Acitretin Isotretinoin
Interference with blood flow in follicular papilla: anticoagulants	Heparin Warfarin
Interference with cholesterol synthesis: lipid-lowering agents	Fibrates (clofibrate, bezafibrate, fenofibrate) Lovastatin
Complexation of zinc (thiol moiety): ACE inhibitors	Captopril Enalapril
Interference with thyroid metabolism: thyrostatics and others	Propylthiouracil Levothyroxine Amiodarone (anti-arrhythmic) Lithium (antipsychotic)

(*continued*)

Table 15.1 (continued)

Telogen effluvium with known or assumed mechanism	
Androgen effect: androgens, anabolics, and progestins with androgenic effect	Mesterolone Testosterone Danazol Nandrolone Stanozolol Norethisterone Levonorgestrel Tibolone
Aromatase inhibition: aromatase inhibitors	Letrozole Anastrozole Formestane
Cytokine effect: interferons	Alpha interferon Gamma interferon
Telogen effluvium with unknown mechanism (in the order of indications)	
Blood-pressure-lowering agents (beta-blocking agents)	Acebutolol Nadolol Atenolol Pindolol Labetalol Propranolol Metoprolol
Topical beta-blocking agents for therapy of glaucoma	Betaxolol Timolol Levobunolol
Analgesics/non steroidal anti-inflammatory agents	Acetaminophen Piroxicam Ibuprofen Indomethacin Ketoprofen Penicillamine Naproxen Gold and gold compounds
Psychotropic agents/antidepressants	Amitriptyline Haloperidol Desipramine Imipramine Doxepin Nortriptyline Fluoxetine Trimipramine Lithium
Antiepileptics	Carbamazepine Paramethadione Clonazepam Phenytoin Ethotoin Trimethadione Mephenytoin Valproic acid

Table 15.1 (continued)

Telogen effluvium with unknown mechanism (in the order of indications)	
Antibiotics/tuberculostatics	Thiamphenicol Isoniazid Ethambutol Gentamicin Nitrofurantoin
Varia	Chloroquine, hydroxychloroquine (antimalarials) Albendazole, mebendazole (anthelminthic agents) Cimetidine, famotidine, ranitidine (antacids) Allopurinol (uricostatic agent) Sulfasalazine (antiphlogistic/sulfonamide) Bromocriptine (prolactin inhibitor/anti-Parkinson agent) Levodopa (anti-Parkinson agent) Halothane (inhalation anesthetic)

cells showing the highest proliferative activity in building up the hair shaft. The abrupt cessation of mitotic activity leads to the weakening of the partially keratinized, proximal portion of the hair shaft and its narrowing and subsequent breakage within the hair canal. The consequence is hair shedding that usually begins at 1–3 weeks and is complete at 1–2 months after initiation of chemotherapy [6]. Alopecia becomes visibly noticeable after the loss of 50% or more of scalp hair. Since normally up to 90% of scalp hairs are in anagen, hair loss is usually copious and the resulting alopecia is quite obvious (Fig. 15.19).

The incidence and severity of chemotherapy-induced alopecia are variable and related to the particular chemotherapeutic protocol, since the intensity and duration of the antimitotic insult are important. Multiple classes of anticancer drugs induce alopecia with frequencies of chemotherapy-induced alopecia (Table 15.1) differing for the four major drug classes: over 80% for antimicrotubule agents, e.g. paclitaxel, 60–100% for topoisomerase inhibitors, e.g. doxorubicin, over 60% for alkylators, e.g. cyclophosphamide, and 10–50% for antimetabolites, e.g. 5-fluorouracil plus leucovorin. Combination therapy consisting of two or more agents usually produces higher incidences of a more severe chemotherapy-induced alopecia when compared with single agent therapy. Chemotherapy-induced alopecia is usually reversible with hair regrowth typically occurring after a delay of 3–6 months [24]. In a number of patients, the regrown hair shows changes in color or structure and texture. In some cases, the hair growth may remain reduced after chemotherapy-induced alopecia [5, 78,83].

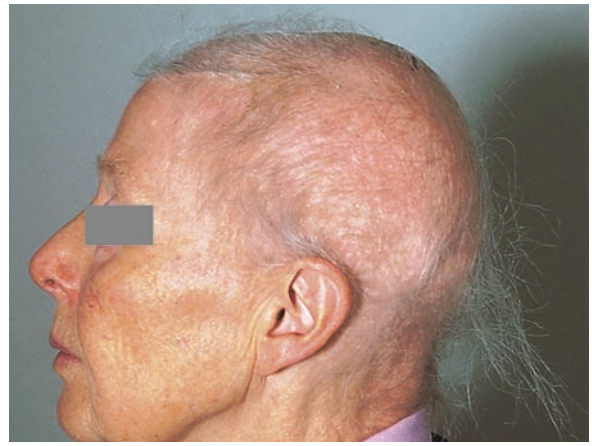

Fig. 15.19 Chemotherapy-induced alopecia

Substantial efforts have led to the development of a number of effective drugs to manage chemotherapy-induced bone marrow suppression and gastrointestinal disturbances. In contrast, progress in chemotherapy-induced alopecia prevention and treatment protocols is still lagging, and no effective treatments for chemotherapy-induced alopecia are available as yet. The major approach to minimize chemotherapy-induced alopecia is by scalp cooling. Unfortunately, most published data on scalp cooling are of poor value [34]. Prerequisite for successful development of strategies for chemotherapy-induced alopecia prevention is the understanding of the pathobiology of chemotherapy-induced alopecia [80]. Several experimental approaches to the development of pharmacological agents are under evaluation and

include: drug-specific antibodies, hair growth cycle modifiers, cytokines and growth factors, antioxidants, inhibitors of apoptosis, and cell cycle and proliferation modifiers [85]. Ultimately, the protection should be selective to the hair follicle, e.g. topical application, such that the anticancer efficacy of chemotherapy is not hampered.

15.7 Multimorbidity

With technologic advances and improvements in medical care, an increasing number of patients survive medical conditions that used to be fatal. This fact combined with the aging of the population means that a growing proportion of patients have multiple concurrent medical conditions. The term "multimorbidity" means several concurrent medical conditions within one person. According to a survey done in Canada in 1998 [23], 30% of the population reported suffering from more than one chronic health problem, and the percentage increased with age. In the United States, the prevalence of multimorbidity among those individuals with the age of 65 and older has been estimated at 65% [88]. Numerous pharmacologic treatments, practice guidelines, and educational programs have been developed for managing chronic diseases. With few exceptions, the interventions address isolated chronic diseases and take little account of the multimorbidity experienced by patients [9, 38]. The increasing number of chronic conditions per patient and the increasing amount of multimorbidity in the elderly population lead to a more complex approach to successful treatment of hair problems in the elderly.

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Aging of Scalp and Age-Related Disorders of Scalp

16

Desmond Gan and Rodney Sinclair

Core Messages

- Scalp, like skin elsewhere in the body, undergoes intrinsic and extrinsic aging processes.
- Bald scalp shows features of both intrinsic and extrinsic aging, typically with marked solar elastosis.
- Hair provides natural ultraviolet protection, but the protection is not complete. Nonbald scalp predominately shows features of intrinsic aging.
- Diseases caused by ultraviolet irradiation are more commonly found in balding scalp, including actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and melanoma.
- Other diseases occurring in the aging scalp include seborrheic keratosis, angiosarcoma, and erosive pustular dermatosis (EPD).

16.1 Introduction

Scalp, like skin elsewhere on the body, undergoes changes with advancing age. Initial changes are considered developmental. These include the enlargement of sebaceous glands at puberty and associated alterations in skin microflora and predisposition to seborrhoic dermatitis. In contrast, later senescent changes are considered to be part of the aging process. Those senescent age-related changes in the scalp that are genetically programmed and due to chronological aging are called intrinsic aging. Those additional age-related changes that are due to accumulated exposure to environmental insults, most notably ultraviolet irradiation, are called extrinsic aging.

Extensive research has already been conducted regarding the characteristics, properties, and mechanism of intrinsic and extrinsic aging of human skin. These observed phenomena in skin aging may also apply to scalp. The presence and severity of hair loss is the principal determinant of the extent of scalp affected by photoaging as scalp hair provides effective natural ultraviolet photoprotection.

16.2 The Appearance of Aging Scalp

The macroscopic appearances of aging scalp are most clearly seen in premature aging disorders. Children with Hutchinson–Gilford progeria syndrome (HGPS) develop thin and lightly pigmented hair progressing to alopecia. There is skin dimpling and mottling, prominent scalp veins, and prominent cutaneous vasculature [62]. Similar changes are seen in the adult onset progeria Werner's syndrome, with alopecia, hair graying, skin wrinkling, facial aging, and appearance of cutaneous malignancies [65].

Changes in the hair are often the most striking aging phenomena on the scalp noticed by people. Graying of hair, weathering of hair shaft, and the balding process (senescent alopecia and androgenetic alopecia) are the three elements that make up the overall appearance.

The aging effects in nonbalding scalp skin are relatively unnoticed. Underneath the hair, the scalp appears

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pale and dry, with certain degree of laxity. In contrast, balding scalp exhibits features of photoaged skin, including irregular pigmentation, wrinkling, severe atrophy, multiple talengiectasia, and findings of cutaneous premalignant/malignant diseases such as actinic keratosis (AK), basal cell carcinoma (BCC), and squamous cell carcinoma (SCC).

16.3 The Aging Scalp Under the Microscope

The most striking difference in the aging scalp when compared with aging skin elsewhere in the body lies on the changes in hair follicles. The risk of developing androgenetic alopecia (AGA) increases with advancing age. There is much debate whether all age-related hair loss is due to the single process of AGA or whether there is a second additive process called senescent alopecia. This debate is described elsewhere in the textbook (see Chaps. 7 and 13).

We were unable to identify any studies comparing the histology of aged nonbalding scalp and Sun-protected skin elsewhere. However, similar intrinsic aging features are likely to occur. The hallmark of intrinsic aging is flattening of the dermal–epidermal junction. Other changes that may be seen include reduced epidermal Langerhan's cells and melanocytes, loss of fibroblast and vascular network, and reduced dermal extracellular matrix [105].

In skin chronically exposed to ultraviolet light, the classical histological change is dermal elastosis, along with deposition of thickened degraded collagen and elastic fibers. These changes were also seen in a study of 140 men affected by AGA and 50 men without AGA. Balding scalp was shown to have thicker dermis, mainly due to the increased level of solar elastosis [72]. This study also showed that the onset of solar elastosis precedes the onset of AGA, meaning that scalp hair does not provide complete protection from the effects of UV irradiation. Other aging features that may also be seen in the Sun-damaged scalp include marked variability in thickness and cellularity in epidermis, unevenly distributed melanocytes, and increased inflammatory cells in the dermis [105].

16.4 The Mechanism of Aging

Most of our understanding of the molecular biology of skin aging is derived from work performed on skin elsewhere on the body and extrapolated to the scalp.

16.5 Intrinsic Aging

16.5.1 Telomeres

Telomeres play an important role in aging, and are probably central to all aging phenomena seen in the body. Telomeres are repeated TTAGGG DNA sequences that cap the end of each chromosome. The 3′ strand extends 75–300 bases beyond the complementary 5′ strand, leaving a single-stranded G-rich overhang at the very end [43]. Looking more closely at its structure, this overhang forms a loop structure by inserting into the proximal telomeric double helix. Telomeric repeat binding factor 2 (TRF-2) encodes a protein that stabilizes this loop structure [28].

With each cell cycle, the DNA polymerase is unable to replicate the outermost end of the DNA, causing progressive shortening of telomeres. This was observed in human fibroblasts where it is shown that the length of telomeres shortens with increasing cumulative population doublings. Also, older individuals' cells have shorter telomeres [35]. Dyskeratosis congenita have shorter telomeres when compared with normal individuals [99]. Oxidative damage toward the GGG residues, produced through UV radiation or cellular metabolism, may accelerate telomere shortening [67].

Both the telomere length and the integrity of its loop structure are believed to be crucial in maintaining the stability of chromosome. When shortening reaches a crucial point, signals are produced to induce cell senescence. Recent observation also demonstrates that shortening of telomere may disrupt the telomere loop structure and exposes the 3′ overhang. This exposure is seen as "toxic" to cells and senescent phenotype is induced. This is shown in a study where cultured fibroblasts are induced into the senescence phenotype when the cells are exposed to T-oligo (nucleotides homologous to the 3′ overhang) [52]. Therefore, it is believed that the progressive shortening represents the internal

clock that determines the onset of senescence of each cell, and therefore plays a pivotal role in the intrinsic aging of skin [43].

Telomerase is an enzyme, which has reverse transcriptase activity (hTERT) that is capable of adding telomeric sequences to the ends of telomere, thereby preventing its shortening [14]. It is expressed in 90% of all tumors [42]. It is found to be present in human epidermis, with downregulation of its level as keratinocytes differentiate into the suprabasal layers [34]. In hair follicle, its presence is highest in the hair bulb containing rapidly proliferating cells [75]. It is not detected in the dermis [34], the cultured fibroblasts, or the melanocytes [14]. The importance of telomerase is highlighted by the study demonstrating premature hair graying, alopecia, and decreased wound healing in mice deficient for telomerase [80]. In Werner's syndrome, the gene product unwinds G-rich DNA structures and is implicated in telomere maintenance [36].

16.5.2 Oxidative Stress

The intrinsic aging of the skin is also driven by the oxidative stress produced by the ongoing cellular mechanism generating the reactive oxygen species (ROS) including superoxide anion, peroxide, and singlet oxygen. In cultured fibroblasts, the increase in the level of ROS is shown to induce the senescene [16]. The multiple mechanisms whereby these particles lead to apoptotic death are accumulated DNA damage, membrane peroxidation, carbonyl modifications, and loss of sulfhydryl groups in proteins. In human scalp skin experiencing graying of hair, anagen scalp hair follicles exhibit features of oxidative stress and apoptotic cells on immunohistochemistry and cell culture. Melanocytic apoptosis was also increased when exposed to additional exogenous oxidative stress [4].

16.6 Extrinsic Aging

Ultraviolet irradiation causes the production of ROS, which activates various cell surface cytokines and growth factor receptors in keratinocytes and fibroblasts – including epidermal growth factor, interleukin-1, insulin, keratinocytes growth factor, and tumor necrosis factor α (TNF-α) [22, 104]. This causes downstream signal transduction pathway. On 4 h of UV irradiation, these signaling kinases in epidermal cells are activated maximally. One of the major downstream effects is the activation of nuclear transcription factor AP-1, composed of the proteins c-Jun and c-Fos [22].

Transcription factor AP-1 is an important member of the process of photoaging as it produces a number of effects. First, matrix-degrading enzymes metalloproteinases (MMPs), such as MMP-1 (collagenase), MMP-3 (stromelysin 1), and MMP-9 (92-kDa gelatinase) are stimulated, which lead to collagen degradation [3]. Second, AP-1 downregulates the expression of procollagen I and III genes, which then interfere with major collagen production [21]. This is achieved by two mechanisms – it binds and sequesters the factors that are part of a transcriptional complex required for procollagen transcription; and indirectly, it blocks the effects of transforming growth factor-β (TGF-β), a major profibrotic cytokine [22]. The level of TGF-β receptors has also been shown to downregulate on UV irradiation, further inhibiting the collagen transcription [73].

In addition, an inflammatory response appears to be an essential part of photoaging. Ultraviolet irradiation activates the transcription factor NF-κB, which induces the expression of pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), TNF-α, and adhesion molecules [106]. These cytokines then feedback positively, resulting in a vicious cycle that amplifies the response of UV irradiation as described before. Furthermore, neutrophils seem to play an important part as they are the initial infiltrating cell type in the dermis following erythematogenic doses of UV irradiation, and also provide a key source of elastase, MMP-1, and MMP-9 [77].

The production of ROS from UV irradiation also directly causes oxidative damage to the upper dermal proteins [82]. Certain amino acids, e.g., tyrosine, arginine, cysteine, methionine, praline, and threonine, are susceptible to oxidative damage. Structures of dermal collagens and elastin are damaged through cross-linking on UV irradiation. These oxidized dermal proteins may result in loss of protein function, loss or gain of enzymic activity, and increased or decreased susceptibility to degradation [104].

Ultraviolet irradiation is also known to cause photoaging through genetic and DNA damage.

Mitochondrial DNA suffers the process of "common deletion," a 4,977 base pair deletion that is detected in many different tissues with advancing age. A study on Sun-protected skin area showed that repetitive UV irradiation for 2 weeks increase the dermal mitochondrial common deletion by up to 40%, and this effect seems to persist in the majority of cases following cessation of UV exposure. In some cases, this common deletion is increased to up to 32-fold after 16 months [9]. Indeed, in a study examining temporal scalp samples, the frequency of mitochondrial DNA deletion in hair follicles is found to increase with age. Subjects with graying of hair also have a higher rate of mitochondrial DNA-deletion, signifying similar processes occurring in the scalp [41].

There is recent hypothesis linking UV irradiation and telomeres in the process of photoaging [43]. As described before, when the loop structure of telomeres is broken, it exposes a toxic 3′ overhang sequence that induces cellular senescence [52]. Ultraviolet irradiation is known to form DNA pyrimidine dimmers, especially on the dithymidine (TT) sites [69]. Oxidative damage from UV irradiation or cellular metabolism causes damage at guanine (G) residues and is shown to accelerate telomere shortening [68]. Therefore, it is possible that telomeres with a structure of repeated TTAGGG sequences are susceptible to ongoing photodamage toward its loop structure, providing a mechanism whereby UV irradiation accelerates the rate of aging caused by telomeres instability.

16.6.1 Other External Factors

Cigarette smoking is another external factor thought to affect skin aging [24]. Cigarette smoking delivers huge range of free radicals and oxidants, and thereby hastening the process of oxidative aging [10]. Smoking is long known to be associated with increased wrinkling independent of the effect of UV light [39]. On Sun-protected skin of smokers, the morphology of the elastic fibers appears to be similar to that observed in solar elastosis [23]. Smoking also induces the matrix metalloproteinases MMP-1 and MMP-3, causing breakdown of collagen [108]. Mitochondrial DNA damage "common deletions" (see above) is found to increase in hair follicles of smokers [54]. Cigarette smoking also decreases rate of wound healing through multiple mechanisms including affecting cutaneous blood flow, fibroblasts functions, and diminished immune response [24].

Menopause as part of aging process of women has profound effects on skin [74]. Estrogen and androgen receptors are found on epidermal keratinocytes, dermal fibroblasts, sebaceous glands, and hair follicles [66]. In human scalp, the predominant estrogen receptors are ERβ [92]. The effects of postmenopausal hypoestrogenemia include reduced level of skin collagen [12] and reduced skin thickness [61]. Estrogen itself also appears to have direct effect on increasing dermal glycosaminoglycan [29] and enhancing vasodilatation in the cutaneous microcirculation of women [53].

Over a lifetime, the scalp may also be exposed to any number of traumatic insults and inflammatory diseases. Prior to the advent of griseofulvin, X-rays were used to epilate the scalp to treat tinea capitis. Dosing errors were common and many children developed long-term sequela from this.

16.7 Seborrheic Keratosis

16.7.1 Epidemiology and Etiology

Seborrheic keratosis is the commonest benign skin tumor in the elderly. Histologically, there is a proliferation of keratinocytes that leads to thickening of the epidermis. The prevalence among adults aged above 50 is in the range of 80–100% [45, 107]. Around 12–23.5% of young adults aged between 15 and 30 are also affected [25, 107]. It is more common among the Whites, affecting males and females equally. The etiology is unknown. Ultraviolet irradiation appears to be an important factor as seborrhoic keratoses are more common in skin areas exposed to sunlight, including face, balding scalp, hands, and upper trunk [45, 107].

Multiple seborrheic keratoses is said to be a familial trait with an autosomal dominant mode of inheritance [76]. Recent genetic studies have identified mutations of fibroblast growth factor 3 (FGFR3) in the range of 39–85% in both familial and sporadic seborrheic keratoses [33, 55] and 19% seborrheic keratoses contain mutation of PIK3CA oncogene (gene encoding for p110α subunit of phosphoinositide 3-kinase) [32].

FGFR3 is a member of the receptor tyrosine kinase family. The same FGFR3 mutation causes severe skeletal dysplasia syndromes such as SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) and thanathophoric dysplasia in the germline [94]. Mutation of PIK3CA oncogene has also been reported in a wide variety of human cancer including breast, colon, ovarian, and gastric cancer. These two mutations are not present in normal epidermal cells, and appear to be functionally important in the development of seborrheic keratoses [32].

Widely accepted as harmless and benign in origin, there are multiple reports of malignant lesions developing within serborrhoeic keratoses, including Bowen's disease, SCC, BCC, and occasionally, melanoma and keratoacanthoma. It is not known whether these are incidental lesions (collision tumor) or malignant transformations [15, 100].

16.7.2 Clinical Features

Seborrheic keratoses on unprotected balding scalp are usually flatter. Lesions initially develop with slightly hyperpigmented sharply defined flat papules (Fig. 16.1). This may be difficult to be differentiated from pigmented solar keratosis, lentigo maligna, or BCC. Seborrheic keratoses then develop into verrucous plaque, and the occurrence of smooth dome-shaped and uneven warty surface with a classical stuck-on appearance. The surface usually has multiple plugged follicles and does not reflect light. Most seborrheic keratoses have fewer hairs on the lesions. They are asymptomatic. Rapid development of multiple small keratoses should alert the possibility of systemic malignancy, most commonly adenocarcinoma of the gastrointestinal tract. This is known as the sign of Leser-Trélat [57].

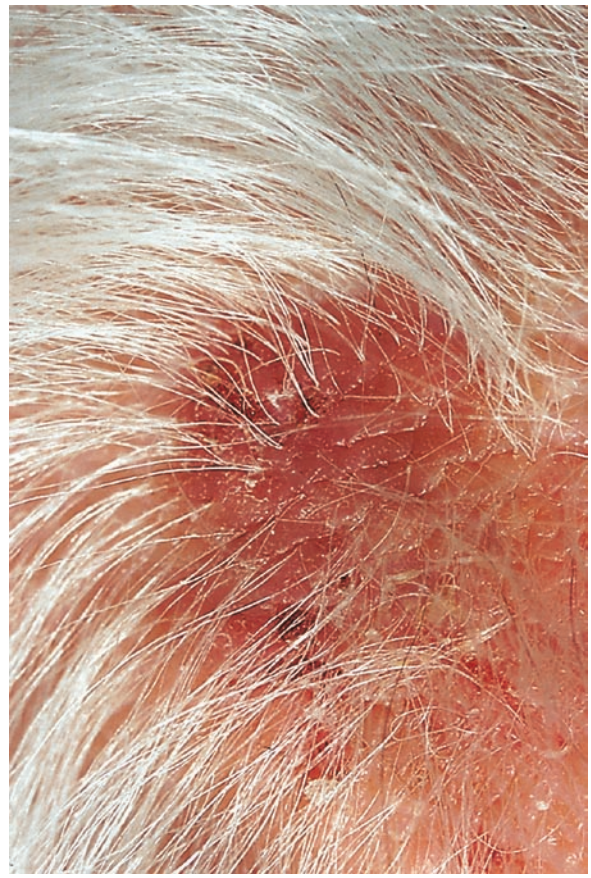

Fig. 16.1 Seborrheic keratosis of the hairy scalp

16.7.3 Histology

There is epidermal proliferation filled with normal keratinocytes that are of basaloid appearance. Some of these cells may contain melanin from the proliferation of melanocytes within the lesion. Common seborrheic keratoses show features of hyperkeratosis, acanthosis, and papillomatosis. Horn cysts from focal keratinization within the lesion or pseudo-horn cysts representing epidermal invagination are commonly seen. One variant called irritated serborrhoeic keratosis show metaplasia and dermal inflammatory infiltrates, and may be mistaken for basosquamous carcinoma [91].

16.7.4 Management

Owing to the benign nature of the disease, treatment is only required for cosmetic purposes or to relieve any symptoms. Lesions that appear atypical or grow rapidly should alert the clinician about the possibility of a malignancy and may require biopsy.

Numerous surgical therapies are available and proven to be effective. These include cryotherapy with carbon dioxide or liquid nitrogen, shave biopsy, laser ablation, electrodessication, curettage, dermabrasion,

or combination such as curettage with electrodessication, or curettage with cryotherapy. It is important to note complications of pigmentation, scarring, and permanent hair loss with destructive therapies. Destructive therapies are also inappropriate where the diagnosis is uncertain and malignancy not excluded. For such cases, histopathological examination is required. While topical fluorouracil was reported to be effective in a case of giant seborrheic keratosis on the frontal scalp, in the authors' experience seborrhoic keratoses rarely respond to this agent [93].

16.8 Actinic Keratosis (AK)

16.8.1 Epidemiology and Etiology

In Australia, the country with the highest prevalence of AK in the world, the overall prevalence in a Caucasian population aged 40 years and above is up to 60% [59]. Chronically Sun-exposed area including balding scalp, face, neck, forearms, and hands are common areas of AK [85].

The single most important cause of AK is UV exposure, especially UVB. Most AKs exhibit p53 tumor suppressor gene mutation secondary to UV damage [48]. The known risk factors include old age, being male, fair skin, latitude, hairstyle, balding of scalp, all of which could perhaps be linked to the end result of increased cumulative dose of UV irradiation [83]. Immunosuppressive state, either in diseases like HIV, in chronic cigarette smoking, or as part of the chronological skin aging process represents an independent risk factor. Human papillomavirus is also implicated in the pathogenesis, possibly in conjunction with UV activation [38]. It is estimated that the annual risk of transformation to SCC is less than 0.1% per year in an Australian study. Twenty six percent of AK resolves spontaneously without treatment, while appearance of new AK yielded a net increase in AK of 22% per year [60].

16.8.2 Clinical Features

AK initially develops from flat dry scaly erythematous patch. With time, it typically develops to become irregularly shaped, well-circumscribed, and variably erythematous. Its fine sandpaper scaliness is better appreciated by palpation than inspection. AKs on scalp are generally not as hyperkeratotic as those found in upper extremities. It is not uncommon to find multiple AK on scalp (field-change) (Fig. 16.2). AK is usually asymptomatic and feels superficial. Signs of induration or pain should always raise a suspicion of progression to SCC.

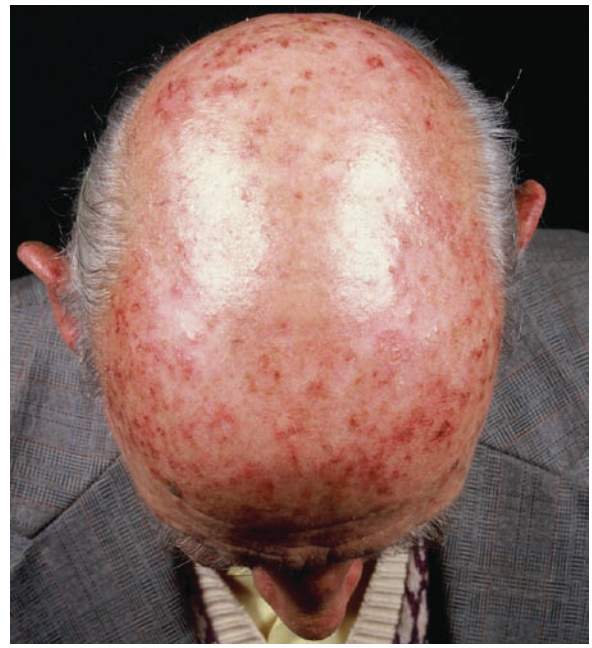

Fig. 16.2 Multiple actinic keratoses of the bald scalp

16.8.3 Histology

In broad terms, the epidermis shows features of dysplasia and architectural disorder; the basement membrane remains intact and the dermis demonstrates chronic Sun damage changes. The epidermis is characterized by alternating columns of hyperkeratosis and parakeratosis within the stratum corneum. The epidermal keratinocytes are pleomorphic with hyperchromatic nuclei, disordered in arrangement and are less basophilic than normal [56]. Areas of atrophy and acanthosis are seen. Basement membrane sometimes becomes irregular with small buds of atypical epidermal cells extending into papillary dermis, signaling the eventual fate of transformation into SCC if left

untreated. The dermo-epidermal junction is flattened. Dermal layer shows solar elastosis with increased inflammatory cell infiltrate and dilated upper dermal blood vessels [101].

16.8.4 Treatment

Prevention with avoidance of sunlight and sunscreen use are obvious measures to reduce the burden of the disease. Multiple topical treatment options are available with proven efficacy to reducing AK. The important considerations when deciding treatment include number of lesions, thickness, tolerability, and practical issues such as availability of patient and cost [19].

16.8.5 Low Number Lesions

16.8.5.1 Cryotherapy

Cryotherapy remains the mainstay of treatment for solitary or discreet lesions, because of its ease of use, low cost, and good clearance rate. The effectiveness of cryotherapy is directly related to the length of freeze time and thickness of AK. In the scalp and face, the total clearance rate was shown to improve from 39 to 83% when freeze time is increased from less than 5 s to longer than 20 s [90]. The freeze time is, however, restricted by the patient's tolerability to pain, and in practice a 5–15 s is appropriate.

16.8.5.2 Curettage and Surgical Excision

This is usually reserved for hyperkeratotic lesions or those that are suspicious of SCC.

16.8.6 "Field Change"/Multiple lesions

16.8.6.1 5-Fluorouracil (5-FU)

5-FU cream is a cytotoxic agent, which inhibits thymidylate synthetase. It works by interfering with DNA and RNA synthesis. Common treatment regime consists of twice daily application for 2–3 weeks. Common side-effects include erythema, local irritation, and pain, and this is usually the limiting factor for duration of course of treatment. A response rate (at least partial clearance of AK) of close to 50% was reported [30].

16.8.6.2 Imiquimod

Imiquimod is an immune response modifier that stimulates innate immunity. It binds to Toll-like receptor and induces cytokine synthesis and release. Local side-effects are similar to that of 5-FU, but the degree of it varies hugely between patients [19]. Normal treatment regimen is 3 times per week application for a duration of 12–16 weeks. This gives a 50% complete clearance and 74% of partial clearance [31]. A shorter regime as follows was demonstrated recently to have similar efficacy [1, 87] – An initial 4-week treatment, with further 4-week treatment if there is no complete clearance on review at eighth week. Meta-analysis showed imiquimod to be superior to 5-FU and cryosurgery for treatment of scalp and facial AK [30]. It has additional benefit of uncovering subclinical AK, which explains the transient increase of AK during initial phase of treatment [20]. However, the main drawback is related to its cost and availability [26].

16.8.6.3 Photodynamic Therapy (PDT)

PDT utilizes topical photosensitizer (e.g., 5-aminolevulinic acid or 5-methylaminolevulinate) with specific dedicated light source to preferentially destroy dysplastic cells. Acute pain during irradiation is a major side-effect. Response rates reported range from 69 to 91% [19]. Its efficacy is proven but comparison studies with other treatments are limited. It provides better cosmetic outcomes when compared with 5-FU or imiquimod [88].

16.8.6.4 Diclofenac Gel

Diclofenac is a nonsteroid inflammatory that inhibits cyclo-oxygenase and reduces prostaglandin production. Increased levels of prostaglandins is seen in photoaging and AK, but the exact mechanism for treatment

of AK is unknown [78]. Treatment of 60–90 days produces a good complete clearance rate of 33–50%. Side-effect profile (mainly pruritus and rash) is better than that of 5-FU or imiquimod [63].

16.8.6.5 Retinoids

Retinoids work by normalizing keratinization and reducing dysplasia. Topical retinoids showed moderate improvement in AK numbers. Oral retinoid is considered as an alternative treatment option for high-risk immunosuppressed patients with a presumed increased risk of malignant transformation from AK to SCC.

16.8.6.6 Other Physical Therapies

Options include ablative lasers, laser resurfacing, dermabrasion, and chemical peels. Although effective, these are rarely performed solely for the purpose of treating AK.

16.9 Squamous Cell Carcinoma (SCC) and Bowen's Disease

16.9.1 Epidemiology and Etiology

SCC is the second most common scalp tumor found on scalp. An Australian study showed that among males, around 5% of SCC is found in the scalp [5]. The incidence among females is much less because of the lower prevalence of hair loss and the protective benefits of hair against development of SCC. Occasionally, multicentric Bowen's disease is found on hairy scalp. Risk factors are similar to that of AK. Thermal scars and radiotherapy (for scalp ringworm and cancer therapy) also increase the risk of SCC, often after a latent period of 20–40 years [86].

Most SCC arise from AK [18], and about 3–5% of Bowen's disease becomes invasive if left untreated [40]. SCC of the scalp is usually discovered early. Risk of cervical nodal and parotid metastasis at time of diagnosis is around 5%, lower than that of pinna or lip SCC. Around 9% of metastatic head and neck cutaneous SCC comes from the scalp [96]. In the scalp, the temporal region appears to spread more readily, probably owing to proximity to parotid and cervical lymph node [96].

16.9.2 Clinical Features

These are commonly found on balding scalp with signs of excessive photodamage. Bowen's disease usually appears as well-demarcated dry scaly patch or thin keratotic plaques. It can be mistaken for psoriasis except that Bowens tends to be fixed in appearance over time or slowly enlarging, while psoriasis evolves its clinical appearance with remissions and relapses. SCC on the scalp may have a variety of presentations including hyperkeratotic plaques, shallow crusty ulcers with an elevated edge (Fig. 16.3), indurated nodules, and cutaneous horns. Induration of what appears to be an AK should alert one to the possible development of SCC.

16.9.3 Histology

In Bowen's disease, there is full thickness epidermal atypia extending down hair follicles and eccrine ducts. Atypical mitoses and an increase in the number of suprabasal mitoses are present. There is loss of granular layer. Overlying parakeratosis and hyperkeratosis is seen. Basal cell layer is intact.

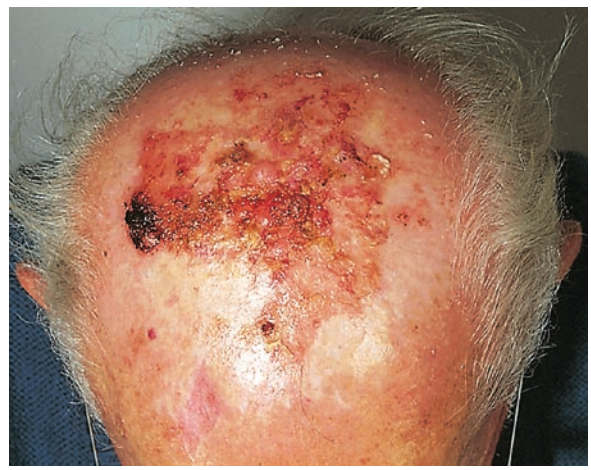

Fig. 16.3 Squamous cell carcinoma (SCC)

In SCC, invasion into dermis with atypical squamous cells arising from the epidermis are seen. There is variable cellular differentiation. Keratinization results in the production of keratin pearls. Histological variants include spindle cell SCC, adenoid SCC, and clear cell SCC.

16.9.4 Treatment

16.9.4.1 Bowen's Disease

A number of treatment options are available for Bowen's disease, and most of these are similar to those of AK. All therapies listed below have approved efficacy (around 10% of recurrence rate), but there is no convincing evidence to date that one is superior to another [64]. Decision depends on size, numbers, location, tolerability of side effects, and patient's fitness. Although surgical excision is regarded as gold standard therapy, there are few RCTs to prove efficacy. Curettage has good evidence of efficacy and a faster healing rate when compared with cryotherapy. Topical treatment wise, PDT seems to be the more effective, less irritating but a more costly treatment when compared with 5-FU. Imiquomod is still under evaluation and may be proved in the near future to be an effective alternative treatment option [17].

16.9.4.2 SCC

Standard treatment for all SCC on the scalp is wide local excision with a margin of 4 mm.

A greater excision margin should be aimed at larger and deeper lesions. In a study looking at cutaneous SCC of head and neck (20% scalp SCC) treated with wide local excision, the reported local recurrence rate with a minimum of 2-year follow-up is 4% [7]. Moh's micrographic surgery offers better treatment outcome and 5-year recurrence rate is reported to be 3.4% [50]. Moh's is reserved for high-risk tumors and high-risk patients. For patients unfit for surgery, other treatment modalities (radiotherapy, PDT, topical 5-FU) may be considered, and appear to be efficient in superficial small SCC.

SCC of the scalp may be unusually aggressive. As the subgaleal plan offers little protection to tumor spread into periosteum, SCC may migrate laterally for great distances [47]. Metastatic SCCs to parotid and cervical lymph nodes have very poor prognosis. Surgical management (parotidectomy, cervical lymph node dissection) with adjuvant radiotherapy is offered in these cases.

Predicting subclinical lymph node metastasis is difficult. "High-risk" features include tumor thickness >4–5 mm, tumor size > 2 cm, histology that shows a poorly differentiated high-grade tumor, perineural invasion, lymphovascular invasion, recurrence after treatment, and host factors such as patients who are immunosuppressed [97]. Imaging to detect subclinical lymph node metastases has low yield. There is no current guideline to support elective treatment of lymph nodes in cutaneous SCC. Experience in sentinel lymph node biopsy (SNLBx) is limited in SCC, but this should be considered in experienced centers for patients with large, recurrent, deeply invasive SCC of the scalp [47].

16.10 Basal Cell Carcinoma

16.10.1 Epidemiology and Etiology

Basal cell carcinoma (BCC) is the commonest scalp skin cancer, especially among the elderly population. While the majority of BCCs occur on the face and neck, around 2% of all BCCs are found in the scalp [5]. Men are more susceptible to developing scalp BCC owing to the tendency to become bald. UV exposure, fair skin, history of radiotherapy treatment for childhood tinea capitis, thermal scars, and immunosuppressive therapy in organ transplant patients (but less significant when compared with the development of SCC) represent risk factors for developing BCC [79].

16.10.2 Clinical Features

Scalp BCCs do not look different from BCCs elsewhere. A typical nodular lesion is a smooth, translucent nodule with superficial talengiectasia. They tend to enlarge slowly, often with central ulceration surrounded by translucent pearly rolled-up edge (Fig. 16.4). Scalp BCCs may be nodular, pigmented, morphoeic, or of the

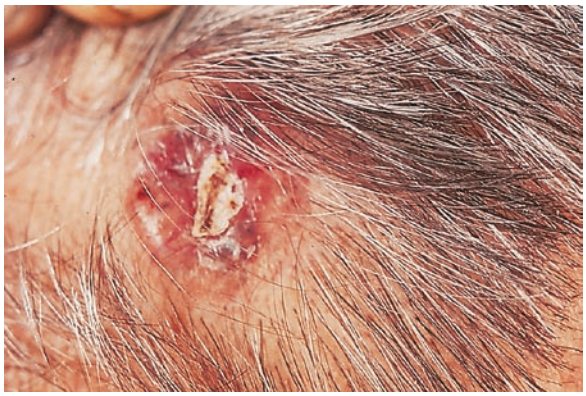

Fig. 16.4 Basal cell carcinoma (BCC)

rodent ulcer type. Because of the tendency to neglect, its existence beneath hair-bearing scalp, BCC can sometimes present as large and deeply penetrating lesions. Fatal intracranial invasion of BCC has been reported in the literature [44].

The risk of BCC metastasis is extremely low, in the range of 0.0028–0.5% [58]. However, most metastatic BCCs originate from the scalp and face region [11]. Characteristically, metastatic BCCs tend to be of extremely large size. The risk of metastasis or death from BCC greater than 10 cm in diameter is reported to be 45% [81]. Metastatic sites include cervical lymph nodes, parotid, submandibular glands, and rarely distant sites beyond the neck via hematogenous channels.

16.10.3 Histology

The tumor cells are found in the dermis, and are seen connecting to the epidermis or a hair follicle. The basaloid (dark blue) appearance is typical, and peripheral palisading is seen where cells are aligned perpendicularly to the basement membrane. There is cleft formation where a separation artifact by mucin shrinkage is created during fixation and staining. Neurotrophic spread predisposing to local recurrence may occur if nerve involvement is seen.

16.10.4 Management

The first-line treatment is surgical excision with at least a 4–5 mm clearance margin. More aggressive lesions, e.g., morpheaform lesions and lesions >2 cm require a bigger margin of 1–2 cm. Five-year recurrence rates are reported between 3.2 and 10%. Moh's micrographic surgery may be offered for high-risk BCCs, and 5-year recurrence rate of primary BCCs is around 1% [49].

Alternative treatment options for BCC include curettage, cryotherapy, laser ablation, and radiotherapy. Radiotherapy appears more efficient than cryotherapy but produces more significant side-effects. Topical PDT and imiquomod are increasingly used for multiple superficial small BCCs but long-term data are required [8].

16.11 Melanoma

16.11.1 Epidemiology and Etiology

Melanoma found in scalp comprises around 1–2% of all melanomas diagnosed, much less than other body sites such as face, trunk, and extremities [5]. Ultraviolet exposure and skin type appear to be the most important risk factors, especially for intermittent nonoccupational sunburn on unprotected balding scalp.

16.11.2 Clinical Features and Histology

Scalp melanomas do not look different from melanomas elsewhere. Head and neck melanoma carries a poor prognosis. Furthermore, scalp melanoma was shown in one study to have the highest rate of local recurrences and the worst survival among head and neck melanoma [51]. In another study comparing the combined neck/scalp melanomas with melanomas elsewhere, the 5-year survival was 83 and 92%, respectively [46]. The poor outcome of scalp melanoma may in part be explained by the vascularity of the scalp, which facilitated hematogenous spread.

Superficial spreading melanoma is the most common subtype found on the scalp (Fig. 16.5), followed by nodular melanoma and then lentigo maligna. The scalp is also a common site for the rare desmoplastic melanoma. Microscopically, solar elastosis is more marked in scalp melanoma when compared with melanoma elsewhere [98].

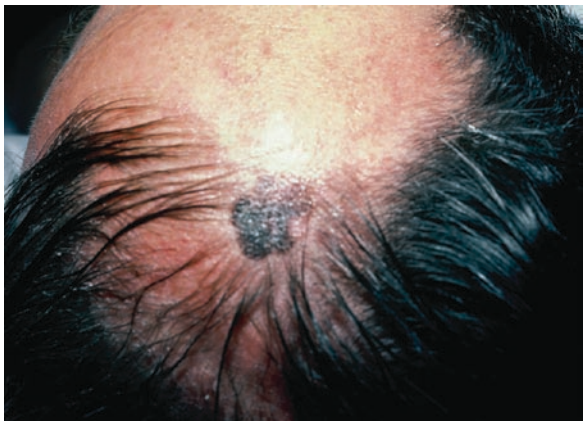

Fig. 16.5 Superficial spreading melanoma

16.11.3 Treatment

A multidisciplinary approach to management of melanoma is important in the management of scalp melanoma. The principle of wide local excision for scalp melanoma is the same as melanoma of other sites, aiming for 0.5 cm margin for in situ melanoma, 1 cm margin for melanoma of <2 mm thickness, and 2 cm margin for melanoma of >2 mm thickness.

Optimal management of regional lymph node in scalp/head and neck melanoma is highly debated. Owing to the complexity of lymphatic drainage, SNLBx in evaluating lymph node spread in scalp melanoma is technically difficult, produces more false-negatives, and has lower identification rates [89]. Nevertheless, it is the most important prognostic indicator [51]. While radical lymph node dissection is commonly advised for sentinel node-positive patients, a survival advantage from performing this procedure is difficult to prove [89].

Postoperative radiotherapy is beneficial for survival and should be offered to melanoma of high-risk local regional recurrence [2]. In addition, adjuvant systemic therapy with high-dose interferon alpha offers better survival for high-risk melanoma patients [102].

16.12 Angiosarcoma

16.12.1 Epidemiology and Etiology

Idiopathic cutaneous angiosarcoma is a rare and highly aggressive malignant tumor of the vascular endothelial cell. It may affect any part of the body, but it most commonly affects the head and neck. The mean age of presentation is 70–80 years [71], with a male:female ratio of 3:1 [6]. It comprises less than 1% of scalp tumor.

Prognosis of angiosarcoma of the scalp is extremely poor. Metastasis to regional lymph nodes and to the lungs has often occurred at the time of diagnosis. Five-year survival rate was reported at 10% [37]. Poor prognostic features include multifocal disease, advanced age, size >5 cm, and most importantly, tumor stage. The significance of histological grading in predicting survival is uncertain [71].

16.12.2 Clinical Features

Angiosarcoma is difficult to diagnose clinically. Most lesions are painless [71]. The earliest lesions often present as ill-defined flat or plaque-like lesions with features of cicatricial alopecia on top, known as "malignant bruise" (Fig. 16.6). More commonly, it presents as single or grouped bluish-red nodules, with or without some degree of alopecia. Progressively, the lesions may involve a large area. Bleeding, ulceration, and gross edema may occur.

16.12.3 Histology

There is often extensive infiltration of tumor to the dermal layer. In the angiomatous form, atypical large

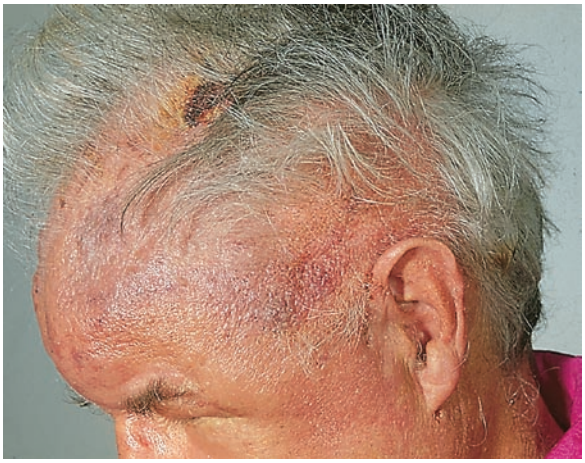

Fig. 16.6 Angiosarcoma

plump endothelial cells form channels of anastomosing dilated vessels extending between the collagen bundles. The solid form is more cellular and contains poorly differentiated spindle and epitheloid cells. Immunohistochemical stains are required to differentiate angiosarcoma from spindle cell SCC or amelanotic melanoma [86].

16.12.4 Management

The mainstay of treatment for angiosarcoma is surgical excision. Preoperative assessments include both CT and MRI of the head and neck for assessment of tumor local extension and cervical lymph node spread. It is often difficult to achieve clear surgical margin because of the extensive local growth. Therefore, surgery involving full-thickness excision including the pericranium with a wide surgical margin of 5 cm is essential. The use of intraoperative frozen section examination and Moh's excision is not shown to improve the surgical outcome [27]. The excision inevitably leaves patients with massive defects requiring grafts for closure. As a huge proportion of excision margins are found to not be adequate on the first excision, a graded reconstruction is recommended so that the final grafting is performed after histology of the excision is obtained.

Radiotherapy has been shown to improve survival rates and should be offered as an adjuvant therapy post resection [71]. The role of chemotherapy is not defined, but agents such as doxorubicin and paclitaxel are shown to have an effect. Gene therapy with intralesional alpha-2b may be used to shrink tumor size.

16.13 Erosive Pustular Dermatosis

16.13.1 Epidemiology and Etiology

Erosive pustular dermatosis (EPD) of the scalp is considered a rare entity. A total of approximately 45 cases have been reported since it was first described in 1979 [70, 95]. However, it is almost certainly underreported. Almost all cases occur in the elderly. The female:male ratio is about 3:2 [70].

The cause of EPD is relatively unknown. It is most commonly found in balding scalp with actinic damage. It is strongly associated with antecedent trauma to the scalp, such as surgical procedures, destructive therapy for actinic keratoses, skin grafting, incidental trauma, radiotherapy, and herpes zoster infection [70, 103]. It does not appear to be caused by bacterial or fungal infections, although bacteria are frequently isolated from cultures [70]. Associations with autoimmune diseases such as rheumatoid arthritis have been reported, but the direct relationship with it is difficult to prove because of the different age of onset. Apart from the scalp and occasionally the face, the other site of occurrence is the legs usually affected by chronic venous insufficiency.

16.13.2 Clinical Features

Patients with EPD typically present with chronic nonhealing lesions. Erythematous crusty shallow erosions are the main findings clinically (Fig. 16.7). Pustules are not always present, and when found, are usually flattened and contain little or no fluid [70]. Beneath the crust, seropurulent material is commonly exposed. The

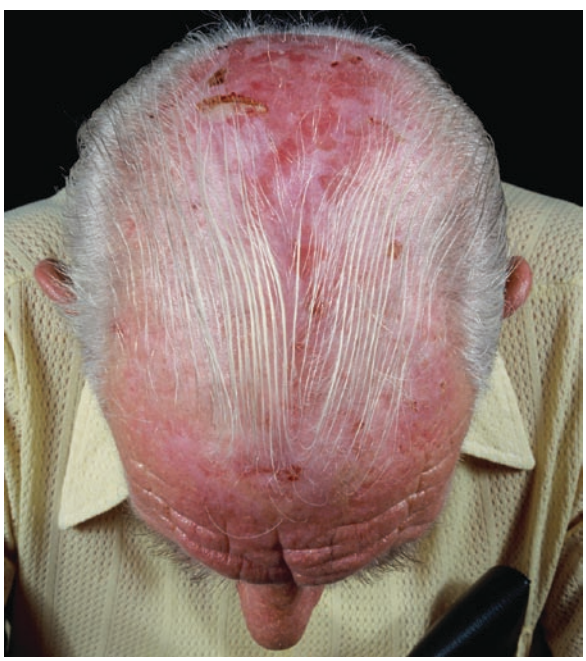

Fig. 16.7 Erosive pustular dermatosis (EPD) of the scalp

surrounding skin looks atrophic. Lesions lead to scarring alopecia. Even though the lesions look inflammatory in nature, there are no systemic symptoms or regional lymphadenopathy. Varying degrees of symptoms from pruritus or mild to moderate pain may be described.

16.13.3 Histology

The histological changes are nonspecific. Chronic inflammatory infiltrates with lymphocytes, plasma cells, neutrophils, and foreign-body giant cells are seen. Dermis is usually thinned. Biopsies taken on the edge of the erosion reveal atrophic epidermis. Hair follicle numbers are decreased and sometimes with features of fibrosis [70].

16.13.4 Management

As the histological and clinical features of EPD are nonspecific, treatment is often delayed. Treatment with high-potency topical steroid is extremely effective, to the point that it is seen as diagnostic of EPD. Topical tacrolimus appeared to be an effective second-line treatment for steroid-resistant cases, or as a steroid-sparing agent [70, 84]. Antibiotics work poorly, regardless of whether there is bacterial growth in the culture. Topical retinoid produced mixed result [70]. Topical calcipitriol was successful in one case report [13]. Surgery is not required once the diagnosis has been made.

Take Home Pearls

- Scalp undergoes intrinsic aging with progressive loss of function from ongoing oxidative stress and genetic insult.
- Ultraviolet irradiation is the most important cause of extrinsic aging in the scalp, especially in the balding scalp. It directly damages the scalp protein structure, causes mutation in epidermal DNA, and activates inflammatory cascades.
- Solar elastosis appears in the scalp before the balding process, signifying that hair does not provide complete protection to UV damage.
- Although seborrheic keratosis is a benign lesion, associated premalignant and malignant lesions may be misdiagnosed, or arise within seborrheic keratosis.
- AK is a common premalignant disease found in balding scalp, with a variety of effective topical treatment options.
- SCC of the scalp sometimes exhibit aggressive characteristics with poor prognosis.
- BCC is the commonest skin cancer in the scalp. Metastasis is extremely rare, but most metastatic BCCs originate from the scalp or face.
- Melanoma of the scalp has a worse prognosis when compared with other melanoma in the head and neck or the body, and may present management dilemma in lymph node management.
- Angiosarcoma of the scalp carries an extremely poor outlook, and requires careful staging before complicated surgery is offered.
- EPD is rare, but when correctly diagnosed responds well to high-potent topical steroids.

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Care of Aging Hair and Photoprotection

17

Zoe Diana Draelos

Core Mesage

- Hair is a natural fiber that can be damaged by photoinduced oxidative damage.
- Sunscreens can prevent damage to hair protein and preserve hair color enhancing cosmesis.
- Aging hair is more susceptible to photoinduced damaged as gray hair lacks melanin, which quenches oxygen radicals from UV radiation.
- Hair growth rate and hair diameter both decrease with advancing age.

17.1 Introduction: Aging Hair

Hair ages differently than other body structures because it is one of the few nonliving tissues that is constantly renewed, the only other similar structure being the nails. Hair care becomes important, as aging progresses because damage to the hair is irreversible and the average hairs on a mature head are 3 years old. This means that the effect of any cosmetic procedure gone awry will be present for a long time. Changes that occur as the follicle matures include a decrease in rapidity of hair growth, a reduction in the diameter of the hair shaft, and a failure to pigment the hair shaft. All these observations are consistent with a loss of follicle vigor. Because hair growth requires high-energy output, this decreased vigor is consistent with the slowing of other body activities with age, but the senescence of the hair shaft is particularly visible. Gray hair is the first sign of maturity noticed by both men and women.

This chapter addresses methods of maintaining the hair in a state of health. Much of the information regarding hair behavior is obtained from the textile literature, especially the processing and dyeing of wool fabrics. Human hair experiences most of the same phenomenon exhibited by wool and extrapolations can be made with accuracy. The topic of discussion will include a grooming regimen for maintaining healthy hair, shampoo selection, conditioner use, and hair photoprotection. These are the major activities that impact aging hair.

17.2 Maintaining Healthy Hair

Healthy hair is full, shiny, soft, and manageable. It resists static electricity, maintains the desired hairstyle, and bounces with movement. Since hair is nonliving, hair care becomes very important, especially with maturity. This section presents ten grooming suggestions to maintain the healthy appearance of aging hair. These ideas are intended to maintain hair beauty despite inevitable follicle senescence.

1. Manipulate the Hair as Little as Possible

There is a misnomer among hair stylists that aging hair requires more chemical processing and more

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manipulation. This is not true. There is no such thing as a "body restoring permanent wave" or a "strengthening hair dye." Increased chemical processing creates more hair shaft damage. Combing, brushing, curling, teasing, and braiding also inflict permanent hair damage. Basically, any manipulation of the hair shaft results in the possibility of cuticular damage, known as "weathering" [19]. Weathering is the sum of chemical and physical environmental insults on the hair shaft, which can be minimized through reduced manipulation.

2. Comb the Hair Gently

One of the most common insults aging hair receives on a daily basis is grooming, which is usually accomplished with a comb. Thus, it is important to select a comb that decreases hair breakage by minimizing the friction between the hair and the teeth of the comb. For this reason, a comb should have broadly spaced smooth teeth, preferably Teflon coated, to reduce combing friction. A comb with close rough edged teeth will grab the hair shafts increasing the chance of hair shaft fracture, usually at the point where cuticular scale is most disrupted or completely absent.

Combing friction is also maximal when the hair shafts are tangled. Unfortunately, the most common reason for combing aging hair is to remove tangles. This means that the hair should be protected from situations where tangles arise, such as exposure to wind or hair teasing. Hair teasing is performed by combing from the distal to proximal shaft against the direction of cuticle overlap. Teasing is used by stylists to cause tangles or "rats" in the hair to create the illusion of volume in mature thinning hair.

3. Select a Vented Ball Tipped Styling Brush

The second most commonly used grooming implement by mature individuals is a brush. The main criterion for brush selection is again friction reduction. Closely spaced natural bristle brushes are commonly selected by mature individuals, but this brush design increases hair breakage. A better option is to select a brush design, known as a blow-drying brush, for general grooming needs. These brushes possess vents or openings on the brush head to prevent heat from building up between the hair and the brush head, preventing heat-induced denaturation of the hair protein. The widely spaced bristles are also plastic and ball tipped to minimize friction. If drawing the brush across the palm of the hand causes discomfort, the brush is not recommended for use on aging hair.

4. Avoid Combing Wet Hair

Hair is more likely to fracture wet than dry. For this reason, it is advisable to gently detangle hair following shampooing from the distal ends to the proximal ends with the fingers, not attempting combing or brushing until the hair is almost dry. Many mature individuals feel that the hair must be styled wet to attain the desired style. This is only partially true. Hair will set in the position in which it is placed the instant that the last water molecule evaporates from the hair shaft. This means that the hair should be styled just before it is completely dry. Thus, it is best to finger detangle the wet hair and allow it to almost dry prior to styling to prevent hair breakage.

5. Air dry Hair and Avoid Heated Appliances

It is common for mature individuals to applying external heat to speed hair drying and to curl the hair shafts. Any form of heat applied to the hair shaft, whether the source is a hair dryer, curling iron, or heated curlers, can permanently damage the protein structure of the hair. Wet hair has water on the outside of the hair shaft and water on the inside of the hair shaft to function as a plasticizer. Hair dryers attempt to speed evaporation of the water on the outside of the hair shaft and heating styling appliances attempt to rearrange the water deformable bonds within the hair shaft. When the hair is rapidly exposed to high temperatures, the water within the shaft turns to steam and exits the hair shaft by creating a loss of cuticular scale, known as "bubble hair." Figure 17.1 is a scanning electron micrograph demonstrating the bubbles created by the energetic steam. Unfortunately, the condition is permanent and bubble hair results in a weakening of the mature hair shaft and eventual breakage.

Many mature patients who present with hair loss may be experiencing hair breakage due to bubble hair. While it is not possible to see bubble hair under a light microscope, it is possible to have the patient collect 4 days worth of hair loss, by placing each day's loss in a separate bag. The dermatologist can examine these bags to determine the ratio of broken hairs without the hair bulb to shed telogen hairs containing the hair bulb. If the number of broken hairs exceeds 20%, the patient is experiencing hair breakage, possibly due to bubble hair. At this point, the dermatologist should inquire as to the use of heated hair drying and styling appliances and make some recommendations. Hair that has been heat damaged appears wavy and friable to the human eye and may possess a burned smell.

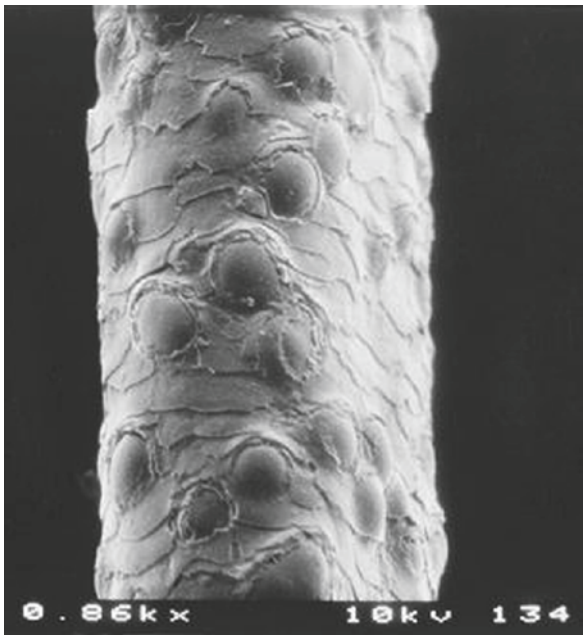

Fig. 17.1 Bubble hair SEM

Even though all forms of heat are damaging to the hair shaft, it is possible to minimize damage by altering the abrupt manner in which the hair contacts heat. Bubble hair is more likely to occur if the room temperature hair shaft is abruptly exposed to high heat. If the hair exposure to the heat is gradual, the damaging effect is not as great. Thus, a gradual temperature increase is recommended. This means that hair dryers can be safely used if the nozzle blowing out hot air is held at least 12 in. from the hair, allowing the air to cool prior to touching the hair shaft. Hair dryers also should be started on low heat to initially warm the hair prior to drying at higher temperatures.

Heat hair rollers and curling irons can be used safely if allowed to cool before application to the hair. These thermostatically controlled devices tend to slightly overheat, which can induce bubble hair immediately on hair contact. Heated styling devices should be unplugged for 1–2 min prior to placing them in contact with the hair. If possible, the styling devices should be operated on a low, rather than high, temperature setting. If the device does not have multiple temperature settings, placing it in a damp towel can lower the temperature of the metal or plastic that contacts the hair. Many patients prefer to use styling heated devices at a high-temperature setting, since the high temperature results in the rearrangement of more water deformable bonds and a tighter, longer-lasting curl.

6. Avoid Scratching the Hair and Scalp

It is not possible to scratch the scalp without scratching the hair. Thus, an itchy scalp, either due to seborrheic dermatitis or postmenopausal itchy scalp, may result in hair loss due to scratching-induced hair damage. It is possible to remove all of cuticular scale from a hair shaft with only 45 min on continuous scratching with the fingernails. Most patients will not scratch their scalp continuously for 45 min, but the hair shaft effects of scratching are additive. Forty-five minutes can easily be accumulated if the patient scratches 5 min a day for 9 days. For this reason, it is important to treat itchy scalp conditions to preserve hair growth.

7. Cut Away Damaged Hair Shafts

Many mature patients are hesitant to cut their hair, since the hair growth rate slows decreasing the maximal achievable length. If the hair shafts have been damaged by too much manipulation and chemical processing, no special shampoo or pricey conditioner can restore hair beauty. For these patients, the overall hair appearance can be improved by removing 1–2 in. from the distal hair shafts. This trims away split ends, formed when the missing cuticle exposes the softer internal cortex, and creates fresh hair ends that are less frizzy, more likely to maintain a curl, and less subject to static electricity. Trimming also eliminates the irregularity of broken hairs that creates the illusion of fuller, healthier hair.

Hair maintenance is an important part of healthy hair, but the products applied to the hair shafts have an equally dramatic effect. Shampoos and conditioners designed to cleanse and beautify the hair require a basic understanding of formulation and application, our next topic of discussion.

17.2.1 Hair Shampoos

Hair shampoos are designed to accomplish the complex task of cleaning all surfaces of each hair shaft, which amounts to a surface area of 4–8 m^2, depending on hair length. Common substances removed from the hair by shampoo include sebum, sweat, desquamated corneocytes, styling products, and environmental dirt [2, 17]. Shampoo was developed because bar soap could produce soap scum when mixed with hard water, leaving behind a sticky film creating scalp pruritus and dull hair [12].

Shampoo functions by employing detergents, also known as surfactants, which are amphiphilic, and other aesthetic ingredients (Table 17.1) [6]. This means that the detergent molecule possesses both lipophilic, meaning oil-attracting, and hydrophilic, meaning water-attracting, sites. The lipophilic site binds to sebum, whereas the hydrophilic site binds to water allowing removal of the sebum with water rinsing [26]. Table 17.2 lists the shampoo detergents currently available to the cosmetic chemist for use in shampoo formulations [20]. Typically, several detergents are combined together to achieve the desired end result. For example, if the shampoo is intended for oily hair, detergents with strong sebum removal qualities are selected. If the shampoo is intended for permanently waved or dyed hair, mild detergents are selected to reduce sebum removal. Aging hair requires proper shampoo selection to cleanse the scalp and beautify the hair.

Table 17.1 Basic shampoo ingredient formulation and function

Detergents Functions to remove environment dirt, styling products, sebum, and skin scale from the hair and scalp
Foaming agents This agent allows the shampoo to suds, since consumers equate cleansing with foaming even though the two are unrelated
Conditioners Leave the hair soft and smooth after sebum removal by the detergent
Thickeners Thicken the shampoo, since consumers feel that a thick shampoo works better than a thin shampoo
Opacifiers Added to make a shampoo opaque as opposed to translucent for aesthetic purposes unrelated to cleansing
Sequestering agents Function to prevent soap scum from forming on the hair and scalp in the presence of hard water. The basic difference between a liquid shampoo and a bar cleanser
Fragrances Added to give the shampoo a consumer-acceptable smell
Preservatives Prevent microbial and fungal contamination of the shampoo before and after opening
Specialty additives Treatment ingredients or marketing aids added to impart other benefits to the shampoo besides hair and scalp cleansing

Table 17.2 Shampoo detergents by chemical category

Alkyl sulfates
Alkyl ether sulfates
Alpha-olefin sulfonates
Paraffin sulfonates
Isethionates
Sarcosinates
Taurides
Acyl lactylates
Sulfosuccinates
Carboxylates
Protein condensates
Betaines
Glycinates
Amine oxides

1. Shampoo Detergents

There are five major categories of shampoo detergents: anionics, cationics, and amphoterics, nonionics, and natural detergents [23]. Each of these groups possessed different hair cleansing and conditioning characteristics. Anionic detergents are the most popular surfactants used in shampoos. They are named for their negatively charged hydrophilic polar group. Anionic detergents are derived from fatty alcohols and are adept at removing sebum from the scalp and hair. The most common anionic detergents are the lauryl sulfates and laureth sulfates.

Most shampoos designed to produce good hair cleansing will contain a lauryl sulfate as the second or third ingredient, with water being the primary ingredient. The detergent listed first is the primary cleanser in highest concentration and the detergent listed second is the secondary cleanser designed to complement the shortcomings of the primary detergent. Examples of lauryl sulfate detergents include: sodium lauryl sulfate, triethanolamine lauryl sulfate, and ammonium lauryl sulfate. These ingredients are popular primary cleansers because they work well in both hard and soft water, produce rich foam, and are easily rinsed. They are excellent cleansers, but must be combined with other detergents, such as the laureth sulfates, in mature persons with reduced sebum production and less physical

activity. Examples of the laureth sulfates are: sodium laureth sulfate, triethanolamine laureth sulfate, and ammonium laureth sulfate.

Two other classes of anionic surfactants are the sarcosinates, such as lauryl sarcosine and sodium lauryl sarcosinate, and the sulfosuccinates, such as disodium oleamine sulfosuccinate and sodium dioctyl sulfosuccinate.

The sarcosines remove minimal sebum and are commonly used in conditioning shampoos or shampoos intended for daily use. The sulfosuccinates are a class of strong detergents useful in removing sebum from oily hair. The mature individual will need to select shampoos with the proper amount of sebum removal for their hair condition. Most persons with seborrheic dermatitis will require an aggressive surfactant, whereas women with colored treated hair will require a mild surfactant. It is the combination of surfactants, which accounts for the different cleansing abilities of the various shampoo formulations.

Table 17.3 lists the remaining types of detergents. The nonionic detergents are the second most popular surfactants, behind the anionic detergents, and bear the name nonionic because they have no polar group. These are the mildest of all surfactants and are used in combination with ionic surfactants as a secondary cleanser [14]. Examples of nonionic detergents currently used in shampoos are polyoxyethylene fatty alcohols and polyoxyethylene sorbitol esters and alkanolamides. Natural detergents, such as soapwort and sasparilla, and cationics are not commonly used, but amphoterics are found in shampoos designed not to sting the eye. The term amphoteric refers to substances that have both a negatively charged and a positively charged polar group. Thus, amphoteric detergents contain both an anionic and a cationic group, which allows them to behave as cationic detergents at lower pH values and as anionic detergents at higher pH values. These unique properties make amphoteric detergents quite unique. Within the amphoteric detergent category, there are several subgroups that include the betaines, sultaines, and imidazolinium derivatives. Cocamidopropyl betaine and sodium lauraminopropionate are the main cleansers in baby shampoos.

2. Conditioning Shampoos

Many mature patients who present to the dermatologist have already severely damaged their hair, and permanent restoration is not possible. Yet, it is important to counsel the patient on how to optimize hair appearance until new growth occurs. One method of minimizing hair damage is to select a shampoo designed to remove sebum and simultaneously beautify the hair, known as a conditioning shampoo. Conditioning shampoos, also known as 2-in-1 shampoos, perform two separate functions based on the difference in shampoo to water ratio during the washing and rinsing phases. During the washing phase, the shampoo is applied to damp hair and massaged into the scalp. In this phase, the shampoo quantity is high and the water quantity is low resulting in detergent cleansing primarily of the scalp, but also the hair. Following completion of the cleansing phase, the shampoo is rinsed from the hair progressively lowering the amount of shampoo and increasing the amount of water. During this rinse phase, a thin layer of conditioner is left behind to coat each hair shaft minimizing damage.

The main conditioning ingredient in conditioning shampoos is silicone, a lightweight clear oil that can coat the hair shaft and smooth the disrupted cuticular scale. Silicone is able to coat the hair shafts without leaving the greasy appearance of sebum. It also reduces

Table 17.3 Shampoo detergent characteristics

Surfactant type	Chemical class	Characteristics
Anionics	Lauryl sulfates, laureth sulfates, sarcosines, sulfosuccinates	Deep cleansing, may leave hair harsh
Cationics	Long-chain amino esters, ammonioesters	Poor cleansing, poor lather, impart softness and manageability
Nonionics	Polyoxyethylene fatty alcohols, polyoxyethylene sorbitol esters, alkanolamides	Mildest cleansing, impart manageability
Amphoterics	Betaines, sultaines, imidazolinium derivatives	Nonirritating to eyes, mild cleansing, impart manageability
Natural surfactants	Sarsaparilla, soapwort, soap bark, ivy, agave	Poor cleansing, excellent lather

combing and brushing friction minimizing hair breakage while restoring shine to damaged hair. Additional cosmetic hair benefits can be achieved by applying a second conditioner after shampooing, the next topic of discussion.

17.2.2 Hair Conditioners

Hair conditioners are liquids, creams, pastes, or gels that mimic sebum by making the hair manageable and glossy [10, 22]. Healthy, undamaged hair is soft, resilient, and easy to disentangle [7], but the trauma caused by shampooing, drying, combing, brushing, styling, dyeing, and permanent waving damages the hair, making it harsh, brittle, and difficult to disentangle [4]. Hair conditioners are designed to reverse this hair damage by improving sheen, decreasing brittleness, decreasing porosity, increasing strength, and restoring degradation in the polypeptide chain. The major uses for hair conditioners in aging hair are discussed next.

1. Hair Conditioner: Major Effects

(a) Decreased Static Electricity

Hair conditioners improve manageability by decreasing static electricity. Following combing or brushing, the hair shafts become negatively charged. These negatively charged shafts repel one another and prevent the hair from lying smoothly in a given style. Conditioners deposit positively charged ions on the hair shaft neutralizing the electrical charge.

(b) Improved Manageability

Hair manageability is another key issue. Manageability refers to the ease with which the hair is combed and styled. The key factor in hair manageability is decreased friction between the hair shafts. Friction is minimized if the hair shafts have smooth even surfaces. This means that the cuticular scales must be present and organized in a tightly overlapping manner. Hair conditioners can reduce friction between hair shafts by as much as 50%, which aids in disentangling [15].

(c) Increased Hair Shine

Healthy hair is shiny due to light reflected by individual hair shafts. Thus, the smoother the hair surface, the more light reflected [18, 25]. Conditioners increase hair shine by increasing cuticular scale adherence to the hair shaft. Large-diameter, elliptical hair shafts with a sizable medulla also maximize hair shine. It is important to note that the medulla is missing from the hair shafts as the hair follicle ages.

(d) Decreased Split Ends

Split ends occur when the cuticle has been removed from the hair shaft and the soft keratin cortex and medulla are exposed to weathering and grooming trauma. The protein of these structures, unable to withstand the damage, splits or frays much like a damaged textile fiber. Conditioners temporarily reapproximate the frayed remnants of remaining medulla and cortex. This strengthens the hair shaft and prevents breakage of the distal ends.

2. Hair Conditioner Selection

The aging follicle produces a smaller diameter hair than the youthful hair follicle. This reduced diameter is termed fine hair, which creates some unique conditioning challenges. Fine hair is particularly prone to weathering and hair grooming damage, since there are more fine hair fibers per weight than coarse hair fibers making the net surface area of fine hair greater. Proportionally more irregular cuticle scales can develop and more of these fine hair fibers are subject to static electricity. Thus, mature individuals should select conditioners developed for fine hair. The primary classes of hair conditioning agents are listed in Table 17.4 [16]. These chemical classes are discussed in greater detail in Table 17.5 [3].

3. Type of Conditioners

(a) Instant Conditioners

Instant conditioners are the most popular among mature individuals. They are applied immediately following shampooing, left on a few minutes, and rinsed. Their primary ingredients are cationic detergents, also known as quaternaries, quaternary ammonium compounds, or quats [1]. Quats deposit a positive charge

Table 17.4 Categories of hair conditioner actives

Alkanolamides
Glycols
Lipids
Protein derivatives
Quaternaries

Table 17.5 Most common hair conditioner formulations

Hair conditioner category	Primary ingredient	Main advantage	Hair grooming benefit
Cationic detergent	Quaternary ammonium compounds	Smooth cuticle, decrease static electricity	Excellent to restore damaged, chemically processed hair
Film former	Polymers	Fill hair shaft defects, decrease static electricity, improve shine	Improve the appearance of dry hair, improve grooming of coarse, kinky hair
Protein-containing	Hydrolyzed proteins	Penetrate hair shaft to minimally increase strength	Temporarily mend split ends

on the negatively charged hair shaft prone to static electricity improving hair manageability. The attraction of the positively charged conditioner to the negatively charged hair shaft also allows the conditioner to remain on the hair following water rinsing [11]. Quats are excellent at increasing adherence of the cuticular scales to the hair shaft, which increases the light reflective abilities of the hair, adding shine and luster. These qualities make them an excellent instant conditioner choice for mature patients with permanently dyed or permanently waved hair where the cuticle has been disrupted as part of the chemical process.

(b) Leave-on Conditioners

A second category of conditioners, known as leave-on conditioners, are applied to the towel dried hair and designed to remain on the hair shafts. These conditioners are based on polymers, such as polyvinylpyrrolidone (PVP), that create a thin film over the hair shaft to fill in defects and create a smooth surface [5]. This increases light reflection improving hair luster and shine in graying hair. In addition, the polymer coating eliminates static electricity due to its cationic nature making the mature hair easier to style.

Some of the film-forming conditioners claim to thicken hair appearance. While consumers may think this means that more hair is present on the scalp, in actuality this claim is substantiated by measuring the diameter of each hair shaft after it has been coated by the polymer film. Indeed the hair shafts have been thickened, but obviously more hair is not present. Leave-on conditioner can help minimize heat damage discussed previously.

(c) Deep Conditioners

The final major category of conditioners is the deep conditioners. Deep conditioners are applied to hair for 20–30 min outside the bath or shower and are available in two types: oil treatments and protein packs. Oil treatments are commonly used for kinky hair, such as African-American hair, that has been straightened. The process of hair straightening results in decreased hair water content, which reduces hair shaft elasticity and predisposes to hair breakage. Applying a heavy oil to the hair shaft is much like moisturizing the skin in that it attempts to both smooth the cuticle and prevent further water loss.

Protein packs represent a second type of deep conditioner. These conditioners are formulated as creams or lotions and remain on the hair for 10–30 min, encouraging penetration into the hair shaft. Protein packs may contain silicones and quaternary ammonium compounds, as well as hydrolyzed protein. Usually, collagen from animal sources is used, but any hydrolyzed egg, beer, avian, placental, or caviar proteins can be used [21]. The protein must be hydrolyzed to a particle size of molecular weight 1,000–10,000 to enter the hair shaft [6]. The source of the protein is not as important as the protein particle size.

As hair weathers, it looses its strength as the cuticular scales are removed and the underlying cortex exposed. This damage creates openings in the hair shaft providing sites for protein diffusion. The diffused protein can increase hair shaft fracture strength by 10%. The ability of protein-containing conditioners to strengthen the hair shaft depends on contact time. The longer the protein conditioner is left in contact with the hair shaft, the more the protein that will diffuse into the shaft. The protein diffusion is reversible, however. This means that any exogenous protein present in the hair shaft will be removed at the time of shampooing necessitating reapplication of the protein conditioner. The end result of using a protein deep conditioner is hair that is softer to the touch, more manageable, shinier, and less subject to static electricity.

17.3 Hair Photoprotection

Photoprotection as it pertains to hair is not a common topic addressed by the dermatologist. After all, hair is nonliving and as such requires no protection from UV radiation because it cannot undergo carcinogenesis. If the hair is photodamaged, it can be removed and replaced by new growth. Thus, at first glance, the whole issue of hair photoprotection might seem superfluous; however, photodamage is one of the most common causes of poor hair cosmesis in an aging population.

1. Hair and UV Radiation

Much of the understanding regarding the effect of UV radiation on hair has come from the textile industry. Natural white fibers, such as wool, cotton, silk, and rayon, turn a light brown/yellow color when exposed to sunlight, a process known as photoyellowing. This same process occurs in human hair when eumelanin and pheomelanin are oxidized to oxymelanin, a photodegradation by-product [8, 13]. Sunlight can also increase scission of the cystine disulfide bonds, which provide hair structural integrity [11]. Thus, sunlight damages both hair color and hair structure.

With advancing age, the natural hair shaft eumelanin and pheomelanin pigments are no longer produced. These pigments function as antioxidants to prevent disruption of the disulfide bonds preserving the strength of the hair shaft. This makes gray hair more subject to UV-induced damage than pigmented hair [9, 24].

2. Hair Sunscreens

Until recently, the main approach to hair photoprotection has been the use of traditional UVB sunscreen actives in formulations designed for hair use, such as instant conditioners, styling gels, and hair sprays. This topical approach is suboptimal because the sunscreen film is not even on every hair shaft and most sunscreens do not adhere well to the hair cuticle. Coating each and every hair shaft with sunscreen without making the hair appear limp or greasy is a challenge no hair care product has overcome. This dilemma has led researchers to question whether photoprotection could be provided through the internal structure of the hair shaft.

3. Enhancing Intrinsic Hair Photoprotection

If eumelanin and pheomelanin provide hair photoprotection, synthetic pigments deposited on the cuticle and within the cortex via hair dyes might also function as sunscreens. Two types of hair dyes can artificially increase hair shaft pigments: semipermanent and permanent hair dyes.

Semipermanent hair dyes are composed of a combination of dyes, such as nitrophenylenediamines, nitroaminophenols, and amionanthraquinones. These dyes are left on the hair 25 min and are used in combination to arrive at the final desired color, which lasts through 8–12 shampooings. Some initial damage occurs to the hair fibers upon dyeing; however, as the hair is exposed to longer periods of UV radiation, the initial damaging effect is outweighed by the antioxidant effect of the color deposited on and in the hair shaft. Thus, white hair exhibits more UV-induced mechanical strength loss than semipermanently dyed hair after 4 days of sunlight exposure. Darker hair dyes provide better photoprotection. The semipermanent dye color is a mixture of reds and blues to create brown. It is interesting to note that the red pigments produce better photoprotection than the blue pigments. This is probably due to the red dyes absorbing the more energetic part of the UV spectrum than the blue dyes.

This same photoprotective effect is also observed with permanent hair dyes. Permanent hair dyes penetrate more deeply into the hair shaft creating color as a result of an oxidation/reduction reaction. They too act as a photoprotectant; however, the permanent hair dyes are more damaging due to the hydrogen peroxide and ammonia used to allow the chemicals to penetrate the hair shaft. More alkaline dyes that allow enhanced penetration also provide better photoprotection. The permanent hair dyes can act as passive photofilters reducing the hair fiber protein damage by attenuating the incident light. This is accomplished by the dye molecule absorbing the light energy, which promotes it to a more excited state, followed by a return to ground state via radiative and nonradiative pathways. Thus, dyeing graying hair provides the best photoprotection currently available.

17.4 Summary

Aging hair requires special cosmetic consideration to account for the lack of pigment and decreased cosmesis. Proper grooming practices and hair care product selection can maximize hair appearance at all ages. The avoidance of improper chemical processing, such

as hair straightening, permanent hair curling, and hair dyeing, can prevent unnecessary weakening of the hair shaft structure. Finally, hair photoprotection is necessary to maintain hair color and strength. This chapter has presented practical hair care ideas for aging hair.

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Possibilities and Limitations for Reversal of Age-Related Hair Loss

18

Nina Otberg*, Isabel Restrepo*, and Jerry Shapiro

Core Messages

- Our modern society faces an increasing life-expectation, and along with this, an increased desire for a youthful appearance.
- Hair plays a major role in our overall appearance, our self-conception and self-esteem. The experience of hair loss is distressing at any age and can reveal our aging process. Aging hair becomes more fragile, is thinner and sparser, and shows decreased growth rates.
- Age-related hair problems include senescent alopecia, androgenetic alopecia, graying, and weathering.
- Treatment option for androgenetic alopecia and senescent alopecia include, among others, topical minoxidil, oral finasteride, and hair restoration surgery.
- Hair loss can also be related to medication, underlying diseases, or surgery.
- Moreover, some inflammatory scalp diseases typically occur in older patients. This chapter discussed the management and treatment option of different forms of age-related hair loss.

18.1 Introduction

Scalp hair plays a critical role in our physical appearance and self-conception. Full, thick, and shiny looking hair is desired at all ages as a sign of youth and health. Most women and men discover some degree of hair loss during the aging process. Age-related hair loss is often referred to as senescent alopecia. However, hair loss in the elderly patient can have multiple reasons, such as medications, surgeries, metabolic disorders, inflammatory processes, genetic predisposition, or a combination thereof. Moreover, aging hair can be a cosmetic challenge, as the hair shafts are thinner and more fragile, and the hair is often dry and brittle. This chapter briefly reviews the common reasons for hair loss in older patients and discusses the treatment options and limitations.

18.2 Senescent Alopecia

Senescent alopecia is described as a very slow, but steady, diffuse thinning of scalp hair starting at the age of 50 years and older [36, 42, 76]. Knowledge about senescent or senile involutional alopecia is meager. Senescent alopecia is believed to be noninflammatory and androgen independent [42]. A normal cycle of the scalp hair involves a long growing period (anagen) with an average length of 2–6 years, a short transitory period of approximately 2–3 weeks (catagen), and a resting period (telogen) of around 12 weeks [41]. Senescent alopecia is characterized by declining growth rates, a decrease in hair shaft diameter, and a prolonged kenogen phase. The latter describes the interval of the hair cycle in which the hair follicle

*Did equal amounts of work.

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remains empty after the telogen hair has been shed and before the emergence of the new anagen hair [6, 17, 27,78]. These changes can also be observed in androgenetic alopecia, but seem to be much more discreet in senescent alopecia.

18.3 Androgenetic Alopecia

The development and occurrence of androgenetic alopecia depends on the genetic predisposition and an interaction of endocrine factors. Androgenetic alopecia or pattern hair loss can start at any age after puberty, and in rare cases, even before puberty [81]. Around 50% of men over 30 years of age are affected by male pattern hair loss, and around 25–38% of women suffer from female pattern hair loss [8].

The degree of male pattern hair loss is classified by the Norwood-Hamilton-scale, which ranges from types I–VIII. Type I represents the prepubertal scalp with terminal hair growth on the forehead and all over the scalp; types II and III show gradual, mostly frontal M-shaped recession of the hair line; types IV–VI show additional gradual thinning in the vertex area; and finally, types VII and VIII show a confluence of the balding areas and leave hair only around the back and the sides of the head. In 1975, Norwood included variations, the middle grades IIa, IVa, and Va, that show a more prominent gradual receding of the middle portion of the frontal hair line and type III vertex, which is characterized by a loss of hair mainly in the tonsure area and a frontotemporal recession that never exceeds that of type III [27, 50]. Female pattern hair loss is classically divided into three stages of severity by the Ludwig classification. It is characterized by a diffuse thinning in the frontoparietal area with a typical preservation of the frontal fringe [46, 53]. However, androgenetic alopecia in women commonly presents with a so-called "Christmas tree pattern," displaying a frontal accentuation of hair loss with or without thinning of the frontal fringe [53]. Moreover, hair loss in women can present in male patterns involving complete frontotemporal recession and vertex thinning [73].

Although patients with pattern hair loss may experience episodes of shedding and worsening, the thinning in androgenetic alopecia is usually a gradual process, which accelerates as the patient gets older. The final degree, age of onset, and expression pattern show great interindividual differences. In the elderly patients, senescent alopecia and pattern hair loss are usually coexistent and the two conditions may be difficult to distinguish. Figure 18.1 shows a 72-year-old female patient with senescent alopecia and androgenetic alopecia presenting in a male pattern with bitemporal recession.

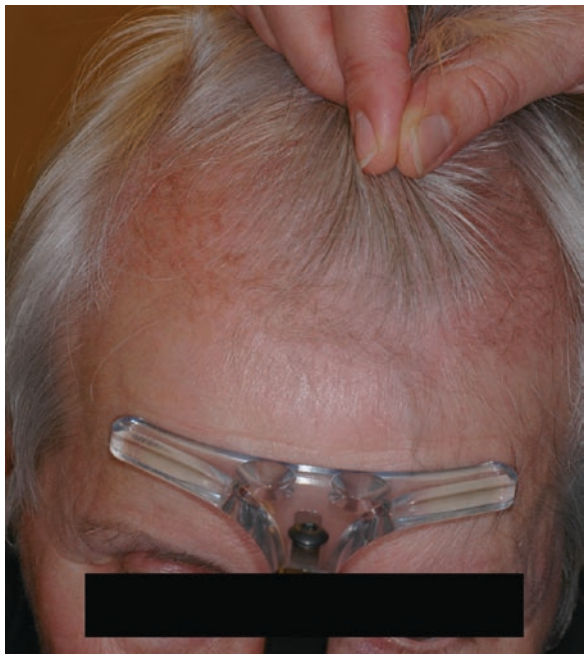

Fig. 18.1 Seventy-two-year-old female patient with senescent alopecia and androgenetic alopecia presenting in a male pattern with bitemporal recession

Androgenetic alopecia is characterized by a miniaturization of terminal hair follicles and their transformation to vellus-like follicles, and along with this, variable bulb depths and shaft diameters accompanied by a change in hair-cycle dynamics. With each hair cycle, the duration of the anagen phase decreases and the proportion of telogen hair increases. As the duration of the anagen phase is the main determinant of hair length, the maximum length of the new anagen hair is shorter than that of its predecessor [22]. With every hair cycle, the affected follicle produces a thinner, finer hair [70]. Moreover, the time between shedding of the hair and anagen regrowth becomes longer, leading to a reduction in the hair present on the scalp, similar to senescent alopecia [17]. Although androgenetic alopecia is not seen as an inflammatory type of hair loss, the earliest histological change is a nonspecific focal perivascular basophilic degeneration in the lower third of the connective tissue sheath, followed

by a mild to moderate perifollicular lymphocytic infiltrate at the level of the sebaceous duct. Multinucleated giant cells may also occur with further progress of the condition [76].

The development of androgenetic alopecia depends, inter alia, on the hair follicle's susceptibility to androgens and the availability of potent androgens, such as dihydrotestosterone (DHT) in the skin. The risk of androgenetic alopecia significantly increases with a positive family history, indicating a strong genetic involvement [15]. The exact mode of inheritance for male and female pattern hair loss is not yet fully understood. Different gene loci have been suggested to be involved in the hereditary transmission of androgenetic alopecia, such as the androgen receptor gene on the X-chromosome [33, 64], the gene locus for insulin-like growth factor-1 on chromosome 12 [48], the cytochrome450c17*alpha* gene on chromosome 10 [13], and the 5α-reductase enzyme genes (SRD5A1 on chromosome 5 and SRD5A2 on chromosome 2) [21, 38]. Recently, a gene locus on chromosome 20p11.22 has been identified to be highly associated with male pattern hair loss [65]. It has been suggested that this gene locus may play a role in an androgen-independent pathway [34].

18.4 Medical Treatment Options for Senescent Alopecia and Androgenetic Alopecia of the Older Patient

18.4.1 Minoxidil

Minoxidil is a piperidinopyrimidine derivative and arteriolar vasodilator, which is known to interact with potassium channels [47]. Laser Doppler velocimetry studies showed an increase in cutaneous blood flow after applying 1, 3, and 5% minoxidil solution to the scalp in 16 balding men, compared with a control group. Five percent minoxidil showed the highest increase in blood flow [89]. Moreover, minoxidil has been shown to upregulate the expression of vascular endothelial growth factor mRNA in human hair dermal papilla cells [44] and increase the proliferation of dermal papilla cells of the human hair follicle, prolonging anagen and preventing cell death with antiapoptotic effects [83]. Another study in balding stump-tailed macaques showed that treatment with topical minoxidil resulted in an increase in anagen hair follicles, reduction in telogen follicles, and an increase in hair follicle size [85]. Histological studies demonstrated an increased in the shaft diameter from 0.029 to 0.043 mm after 12 weeks of treatment [32].

Additionally, minoxidil seems to have an immunoregulatory effect. In vitro studies showed a suppressive effect on human T-lymphocytes [25], supported by histological findings of a reduced perifollicular infiltrate [88] and mild to moderate efficacy in the treatment of alopecia areata [37]. Minoxidil is probably catalyzed in the lower outer root sheath by minoxidil sulphotransferase to minoxidil sulfate, the active metabolite, which is believed to stimulate hair follicles [12, 19].

Minoxidil 5% is approved by the US Food and Drug Administration (FDA) for the treatment of androgenetic alopecia in men, and 2% is approved for the treatment of female pattern hair loss. Topical application of 1 mL or 25 drops of 5% minoxidil scalp lotion twice daily or half a cap of 5% minoxidil foam to the dry frontoparietal and vertex scalp is recommended; the lotion should stay on the scalp for at least 4 h, and studies have shown 75% of absorption during this time period [51].

In a clinical trial in 636 men and 630 women, a therapeutic benefit of topical 2 and 5% minoxidil solution was compared with age, duration of balding, and size of the balding vertex area in men, as well as age and duration of hair loss in women [69]. In general, younger subjects showed a better response than older subjects. Men with a duration of hair loss for <5 years showed a greater effect than those with a balding duration of >21 years. In women, no correlation between the duration of balding and response to minoxidil could be shown. Men with a balding vertex area of <5 cm in diameter showed a greater treatment effect than subjects with more pronounced vertex balding (>15 cm). Duration of hair loss of <1 year when compared with >10 years at the onset of treatment resulted in a considerably more effective treatment with respect to stabilization of alopecia and new hair growth.

The most common side effect is symmetrical facial hypertrichosis, reported more in women than in men [61], allergic contact dermatitis, worsening of seborrheic dermatitis caused by propylene glycol [24], and tachycardia secondary to decreased blood pressure. Patients with heart problems should be cautious, and in case of doubt, should use the medication only with approval from a cardiologist [74].

The mechanism of action of minoxidil is androgen-independent, and therefore, may work for both senescent alopecia and androgenetic alopecia. However, its efficacy is limited in long-standing alopecia, which is more likely seen in the elderly patients. The risk of side effects of topical minoxidil can be regarded as fairly low. Even though older patients may have an increased risk of tachycardia or arrhythmia, minoxidil can be recommended for patients with senescent and/or androgenetic alopecia. Treatment should be tried for at least 6 months to 1 year. Measurements of hair density and hair shaft diameters before and after 1 year of treatment help to determine the efficacy and can help both the physician and the patient to decide whether the treatment should be continued indefinitely. Figure 18.2a shows a 68-year-old female patient before and after 6 months of treatment with topical minoxidil (5% solution of 1 mL twice daily).

18.4.2 Finasteride

Finasteride binds irreversibly to the 5α-reductase isoenzyme 2 and inhibits the conversion of testosterone to dihydrotestosterone, resulting in a decrease in serum and scalp DHT levels, while increasing the scalp levels of the testosterone [65]. Finasteride has been used for the treatment of male pattern hair loss since 1997.

Finasteride can stabilize hair loss in 80% of patients with vertex hair loss and 70% of patients with frontal hair loss. The chance of mild to moderate regrowth is 61% on the vertex and 37% on the frontal scalp [9, 91]. After 24 months of continuous use, 66% of the patients experienced a certain amount (approximately 10–25% of the hair that the patient lost previously) of hair regrowth in the vertex area [57, 91]. The treatment should be started as soon as the patient notices hair thinning, as hair regrowth is limited. However, finasteride has shown an improvement in older men with longer standing bald areas. A small 24 months double-blinded placebo-controlled study on 28 men aged 53–76 years taking finasteride 5 mg/day for benign prostatic hyperplasia showed statistically significant improved hair counts in a circular balding 1 in. target area, when compared with a placebo group [11]. About 39% of men aged 41–60 years on finasteride of 1 mg daily vs. 3% on placebo showed a significant improvement in hair density after 2 years [93].

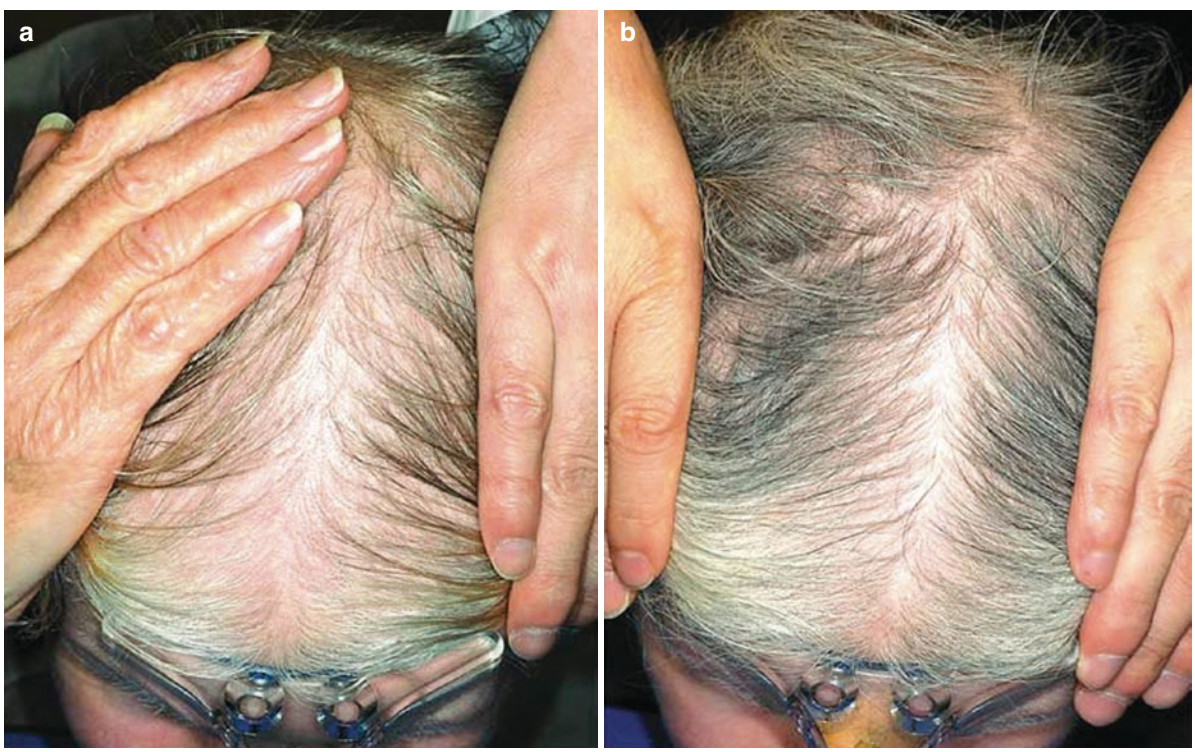

Fig. 18.2 Sixty-eight-year-old female patient (**a**) before and (**b**) after 6 months of treatment with topical minoxidil (5% solution 1 mL twice daily)

Therefore, older patients with androgenetic alopecia without benign prostatic hyperplasia should take 1 mg finasteride daily.

The efficacy of finasteride in women with androgenetic alopecia is controversial. In a double-blind, placebo-controlled multicenter trial, oral finasteride of 1 mg/day for 1 year did not promote hair growth or slow down the progression of hair loss, and did not improve follicular counts in horizontal sections of scalp biopsies in postmenopausal women with female pattern hair loss [63, 93]. Iorizzo et al. showed an improvement in hair density in premenopausal women 6 months after treatment with oral finasteride of 2.5 mg in combination with an oral contraceptive containing drospirenone and ethinyl estradiol [39]. Trueeb et al. reported successful treatment of patterned hair loss with 2.5 or 5 mg/day of oral finasteride in five postmenopausal women without clinical or laboratory signs of hyperandrogenemia. Improvement in hair growth was observed as early as 6 months of therapy [82].

Oral finasteride at a dose of 1 mg/daily is FDA approved for men with androgenetic alopecia, and has been shown to be effective in prevention and treatment of hair loss [67].

Finasteride is found to be well tolerated with side effects occurring in fewer than 2% of patients [40, 45]. The side effects included decreased libido in 1.8% of the recipients vs. 1.3% in the placebo group, erectile dysfunction in 1.3% of the recipients vs. 0.7% in the placebo group, and decreased ejaculate volume in 0.8% of the recipients vs. 0.4% in the placebo group [48, 62]. Effects on spermatogenesis remain controversial. A randomized, double-blind placebo-controlled study in 181 men with androgenetic alopecia taking finasteride of 1 mg or placebo for 48 weeks, found no important effects on sperm concentration, total sperm per ejaculate, sperm motility, or sperm morphology [58]. Another study found a significant drop in sperm count by 34% after 26 weeks of finasteride of 5 mg/day, but the changes became smaller with time and were insignificant by week 52. No effects on sperm morphology were found [2].

Levels of prostate-specific antigen (PSA) can be decreased by around 50% with finasteride therapy [18]. Patients of 40–49 years of age had a median decrease in serum PSA of 40% and patients of 50–60 years of age had a median decrease of 50%. Therefore, a baseline PSA should be taken in men over 40 years, and the family doctor should be advised to double the PSA value while the patients are taking finasteride [9].

Results from a large prostate cancer prevention trial including 18,882 male patients of 55 years of age or older with benign prostate hyperplasia showed a 24.8% relative reduction in the prevalence of prostate cancer after 7 years of continuous use of oral finasteride of 5 mg daily, when compared with a placebo group [79].

Unusual side effects like gynecomastia, mastalgia, exfoliative dermatitis, perioral numbness, swollen lymph nodes, and depression have been reported and resolved with the drug cessation. Finasteride is metabolized in the liver. No interactions with other medications have been recognized.

Finasteride is not approved for women. Few data are available on the safety of oral finasteride in women. While no significant side effects have been reported so far, short- and long-term side effects may still be encountered and hence are unknown. Women of childbearing age should be advised about contraception. Oral finasteride should not be used in women with a positive personal history of breast or ovarian cancer, and should be used cautiously in women with a positive family history of breast or ovarian cancer.

Finasteride is a specific treatment for androgenetic alopecia. Older patients with pattern hair loss may experience a mild to moderate improvement in hair density and an arrest of further loss. Finasteride most likely has no effect on senescent alopecia, as the underlying pathogenesis seems to be androgen independent.

18.4.3 Dutasteride

Dutasteride is an inhibitor of 5-α reductase isoenzymes type 1 and 2; it is three times as potent as finasteride in inhibiting 5-α reductase isoenzyme type 2 and more than 100 times as potent in inhibiting the type 1 isoenzyme [16]. Dutasteride can decrease serum dehydrotestosterone by around 90% and increase the levels of testosterone in serum and scalp [68].

Dutasteride has been approved by the FDA for the treatment of benign prostate hypertrophy at a dose of 0.5 mg daily.

A case report of a 46-year-old woman who had limited improvement from finasteride showed signs of hair regrowth and thickening 6 months after the treatment with dutasteride of 0.5 mg daily and oral contraceptive pills; after 9 months of treatment, the clinical

diagnosis of androgenetic alopecia could no longer be made [56]. Phase II trials in 416 men showed that dutasteride of 2.5 mg exhibited better results in hair density at weeks 12 and 24, when compared with finasteride of 5 mg [55].

The more important side effects are feminizing effects on male fetuses in pregnant woman, decreased libido, impotence, decreased sperm count, semen volume, and reduction in semen motility, but no significant changes in sperm morphology. Men under treatment with dutasteride should not donate blood until they have been off the medication for at least 6 months; this prevents administration of the medicine to a pregnant female transfusion recipient.

Long-term safety of dutasteride in young men and women in general is not entirely known. Owing to the long half-life of approximately 5 weeks, serum concentrations remain detectable for up to 4–6 months after discontinuation of treatment with dutasteride, and possible side effects may also remain for several months. Dutasteride should be reserved for exceptional and therapy-resistant cases. More studies are needed to decide if dutasteride is safe and efficacious for the treatment of androgenetic alopecia in older men and women.

18.4.4 Low-Level Light Therapy

Laser sources have become very popular in medical and nonmedical settings. They guarantee hair growth and are available without prescription. While the mechanism of action is unknown, one theory suggests an increased blood flow at the dermal papilla [10]. Another possible mechanism involves the paradoxical growth of hair that occurs in patients undergoing laser hair removal [1]. One laser device, the HairMax Laser Comb® (Lexington international, LLC, Boca Raton, FL), is approved by the FDA as a medical device. Treatment protocols include 15–30 min treatments on alternating days for 2–4 weeks, tapering to 1–2 treatments per week for 6–12 months, followed by biweekly or monthly maintenance treatments. Some authors report a change in the texture and quality of hair in patients using laser comb devices even if there is no detectable regrowth [5]. Low-level laser sources appear to be safe in the treatment of hair loss. There have been no blinded studies published in the peer-reviewed literature on hair regrowth, density, and thickness. More studies are needed to determine if low-level laser light is reasonably effective and safe in the treatment of hair loss in men and women of any age.

18.4.5 Hair Restoration Surgery

Hair transplantation is an excellent treatment option for patients with androgenetic alopecia, especially if an improvement in hair density in certain areas is desired. Optimal candidates for hair transplantation are those with a high hair density in the occipital donor area, those with a moderate extent of alopecia, and those without contraindications for surgery [84]. Before the patient and physician come to the decision to perform hair restoration surgery, other causes of temporary or permanent alopecia should be eliminated. Hair line design, evaluation of the donor and recipient areas, as well as the discussion of graft numbers are the basic parts of the hair transplant consultation. Senescent alopecia can lead to a limited donor hair supply in the elderly patient. However, senescent alopecia is not a contraindication for hair restoration surgery, as long as the patient has a realistic expectation regarding the treatment results. Hair restoration surgery is ideally combined with medical treatment to prevent further loss and to improve hair growth, density, and texture.

There are two donor hair harvesting techniques that can be performed under local anesthesia. In one technique, a strip of scalp skin is taken from the occipital area, which is then divided into mini- or micro-grafts, each containing 1–4 hairs. The grafts are than planted into tiny slits in the desired recipient area. The other harvesting technique is follicular unit extraction: multiple follicles are harvested with small 1 mm punches and finally planted in the target area. The last-mentioned technique avoids a long linear scar in the occipital area. However, this technique is more labor-intensive and therefore usually more expensive. A natural-looking result can be achieved with both the procedures; one or two sessions can usually provide a good coverage of a balding recipient area. The final results are usually seen in 6–8 months after the surgery. Figure 18.3a, b shows a 66-year-old female patient before and 7 months after one session of hair restoration surgery. The patient received a total number of 1,145 micrografts containing 1–3 hairs per graft.

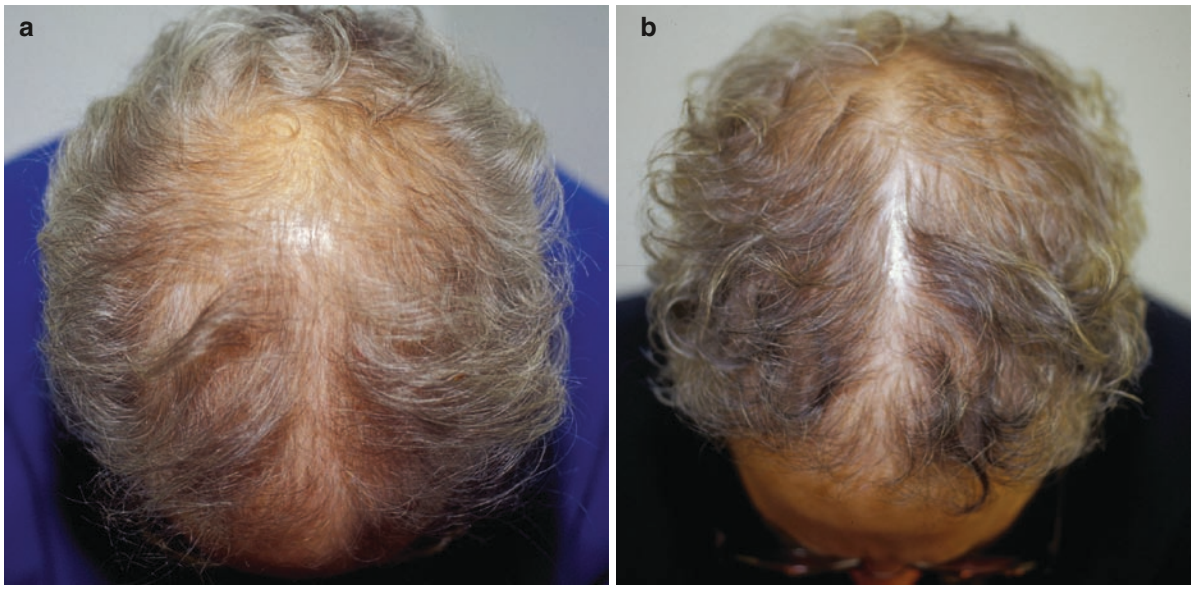

Fig. 18.3 Sixty-six-year-old female patient before (**a**) and 7 months after one session of hair restoration surgery (**b**)

Complications include infection, facial edema, permanent scalp dysthesias, keloid formation, neuroma, A-V fistula formation in <1% of patients, and temporary hair loss in the donor or recipient area.

18.4.6 Wigs and Hairpieces

Natural-looking wigs or hair pieces can have a significant, positive impact on a person's appearance. Especially, if medical therapy fails or is contraindicated and if the patient is not a candidate for hair restoration surgery, a wig or a hair piece should be considered. Patients can choose from a great variety of styles and different types of mountings. In general, well-made hair pieces with real human hair look more natural than full wigs; custom-made, ideally from human hair, wigs look much better than synthetic wigs. The quality of real human hair shows great variation: Asian hair (usually from China or India) can be used processed or unprocessed and is usually less expensive, but also less natural-looking than European hair in a Caucasian patient. Frequently, a combination of synthetic hair and natural hair is used in wigs. Custom-made wigs with human hair are far more expensive and take more effort in maintenance when compared with synthetic wigs. The acceptance of a hair piece or a wig is different in men and women, as well as in different ethnic groups. Women usually can accept the idea of wearing a wig or hair piece better than men. African-American women are in general less hesitant to wear wigs, when compared with Caucasian women. It may appear unusual for a man in his 60s or 70s with long-standing androgenetic alopecia to suddenly change his appearance with a hair piece. Men usually make the decision to wearing a toupee earlier in life. However, wigs and hair pieces can provide excellent scalp coverage in both men and women with androgenetic alopecia, senescent alopecia, or other hair-loss conditions at any age.

18.4.7 Cosmetic Agents

Aging hair creates a cosmetic challenge in many different ways. Certainly, one major concern of aging hair is graying; moreover, aging hair becomes thinner, more fragile, dry, and brittle. Unpigmented hair is more susceptible to UV-induced damage than pigmented hair, indicating that the color granules are providing some protection from oxidative damage [80]. Thus, white hair and advanced gray hair are more susceptible to the damaging effects of UV

radiation than pigmented hair. Even though hair dyes are, in general, in some way damaging to the hair shaft, the photoprotective effects of replacing hair shaft pigments may offset some of this damage. Theoretically, the darker the hair dye color, the more photoprotection should be achieved. Different types of hair dyes are available. Semipermanent hair colors are a mixture of dyes designed to create the desired final color for the duration of 1–4 weeks. Permanent hair dyes penetrate more deeply into the hair shaft; the color is created by an oxidation/reduction reaction. Permanent hair dyes remain in the hair shaft and are potentially more damaging as a result of the hydrogen peroxide and ammonia used to allow the chemicals to penetrate the hair shaft [20]. Dyed hair can change a person's appearance drastically and provide a more youthful look. Hair dyes are more commonly used by women. In men, gray temples are cosmetically more accepted; moreover, it may be difficult to create a natural-looking transition from dyed scalp hair to gray beard hair or side burns. Current research activities focus on selective topical liposome targeting melanin, genes, and proteins to hair follicles for therapeutic and cosmetic modification of hair [35].

Photoprotection and protection against heat damage can be provided by different styling products, such as blow drying conditioners, styling gels, serums, and hair sprays.

Elderly patients frequently tend to wash their hair less frequently. This can lead to scalp problems, such as seborrheic dermatitis, and especially, if the hair tends to be greasy, with a flat and less full appearance. Frequent shampooing is recommended; this will leave the hair fluffy and give the illusion of thicker hair. The use of conditioners with every shampoo is recommended, as the conditioner leaves a thin coating on the hair shaft, providing a shiny, healthy look; also, the use of conditioner makes the hair more manageable and less susceptible to further damage. A deep protein conditioner can be used once weekly, especially if the hair is dry and longer than 10 cm.

Apart from different cosmetic product, the hair style itself can considerably change a person's look. A straight part should be avoided, as it makes thin hair look sparser. Layered haircuts can give the illusion of fuller hair and allow the patient a longer hair style [83]. Permanent waves, preferably with big rollers, can make the hair feel thicker and impart more body.

18.5 Other Noninflammatory Causes and Treatment Options for Hair Loss in the Aging Patient

18.5.1 Telogen Effluvium

Telogen effluvium is a common cause of hair loss in older patients. Women are far more often affected than men. Women often times present with the "bag sign" by bringing in bags of hair that they have collected every day or over a couple of days. Telogen effluvium can be acute with a sudden onset or chronic, with recurring episodes of shedding over many years. The most common causes of acute telogen effluvium in older patients are medications, surgery, thyroid disease or other metabolic disorders, high fever, and major emotional stress. Telogen effluvium in premenopausal women is frequently seen in iron deficiency, after childbirth, and after diets with rapid weight loss. Chronic telogen effluvium may be caused by multiple triggers; however, in many patients with prolonged hair loss, the triggers are difficult to identify [73, 90]. In telogen effluvium, anagen hairs are prematurely shifted into telogen hairs and the normal anagen/telogen ratio of 90:10 can switch to 70:30. Patients with telogen effluvium can shed more than 300 hairs per day. The shedding usually starts around 3 months after the initiating event or trigger [29, 73, 96]. Shedding can usually be noted on the entire scalp; hair thinning may be diffused or more pronounced in the temporal area. The hair pull test is usually positive on the entire scalp or sometimes only in the occipital area. A scalp biopsy can help to confirm the diagnosis and to identify other concomitant hair disorders, such as androgenetic alopecia and/or senescent alopecia.

Treatment of telogen effluvium can be difficult, as the removal of the cause is the major treatment goal. It is important to take a thorough medical history, which should include every medication taken. If the shedding was first noticed 2–3 months after starting a new drug, it should be discussed with the patient and the family physician whether the medication should be discontinued or changed to an alternative product. Low-iron levels are usually not a major concern in postmenopausal women, but ferritin and iron levels can be low in elderly patients with hematological diseases, poor diet, or cachexia. Borderline hypothyroidism can be difficult to identify. Patients who complain about hair loss,

depression, lack of energy, mental fatigue, cold intolerance, weight gain, and/or constipation are suspects for the diagnosis of hypothyroidism. TSH levels may fluctuate but are usually elevated, with normal or reduced thyroid hormone levels. If a thyroid dysfunction is suspected, the patient should be closely followed by an endocrinologist.

Topical minoxidil solution, 2 or 5% in women, 5% in men, 1 mL twice daily, can be helpful in the treatment of telogen effluvium, especially in patients with prolonged hair loss with unknown triggers or in patients with drug-related hair loss who are unable to discontinue the drug.

18.5.2 Anagen Effluvium

Dystrophic anagen effluvium usually occurs in patients after chemotherapy, radiation, or in rare cases, after exposure to toxins or owing to alopecia areata. Anagen effluvium is a result of a disturbance of hair follicle matrix cells. The anagen phase is interrupted and the hair falls out 7–14 days after the initiating event without entering catagen or telogen. Once the initiating trigger is removed, the hair usually regrows after around 120 days. The use of antioxidants to prevent anagen effluvium has been discussed, but the evidence for its efficacy is lacking. Cases of incomplete recovery following multiagent chemotherapy have been reported [75]. Patients should be advised about scalp prostheses and other forms of head dresses.

18.6 Inflammatory Cause and Treatment Option of Hair Loss in the Aging Patient

18.6.1 Alopecia Areata

Alopecia areata is an inflammatory, nonscarring hair-loss condition with a lifetime risk of 1.7%. It is considered to be an autoimmune disease and its pathogenesis has mainly been explained as an inappropriate immune response to hair follicle-associated antigens. Both the genders are equally affected [71]. Alopecia areata usually presents with round, well-demarcated patches of hair loss and is reversible in most cases. The condition can start at any age; however, patches usually occur at a younger age for the first time. Figure 18.4 shows a female patient presenting with her first episode of alopecia areata at the age of 62. The course of this condition is unpredictable. Once the patient has developed one episode of hair loss, the patient is likely to develop another episode of alopecia areata at some point in his or her life. Patients with alopecia areata have a 5% risk of losing their entire scalp hair (alopecia areata totalis) and a 1% risk for a total loss of body and scalp hair (alopecia areata universalis) [73]. Alopecia areata can present in a diffuse generalized pattern, which can subsequently lead to alopecia areata totalis or may just present with moderate diffuse thinning (alopecia areata incognita), which can easily be mistaken for androgenetic alopecia, senescent alopecia, or telogen effluvium. Acute alopecia areata usually presents as a dystrophic anagen effluvium. The hallmark of acute alopecia areata is exclamation point hairs, which occur as a result of the disturbance of hair-shaft formation. The hair shaft breaks off at the skin surface (black dots) and is pushed out of the follicle; the tip is usually dark and wider when compared with the thin, light proximal part (exclamation point hair). Patches of alopecia are usually symptomless, but can show a mild, peach-colored erythema, and may be accompanied by mild to moderate itch. The hair pull test, in an active disease stage, is usually extremely positive.

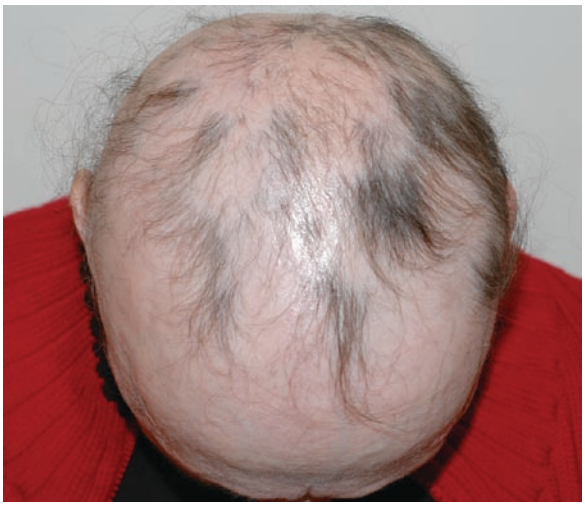

Fig. 18.4 Female patient presenting with her first episode of alopecia areata at the age of 62

Histopathological features of alopecia areata show a typical peribulbar lymphocytic infiltrate, generalized miniaturization, and a marked increase in catagen and telogen hair follicles [92]. Alopecia areata shows a high rate of spontaneous regrowth. It is believed that 80% of patients regrow their hair within 1 year without any treatment. Standard treatment for patchy alopecia includes intralesional and topical corticosteroids. For more advanced stages and therapy resistant cases, topical corticosteroids under occlusion, topical immunotherapy with diphenylcyclopropenone or anthralin are the treatment options of first choice. Other therapeutic options include psoralen UVA therapy, systemic corticosteroids, other oral immunosuppressants, or 308-nm excimer laser. Minoxidil 5% solution can be used as a concomitant treatment, but has not shown to be effective as a monotherapy in alopecia totalis or universalis.

18.6.2 Primary Cicatricial Alopecias

Inflammatory cicatricial alopecias are a diverse group of rare scalp disorders that can be divided into three main groups based on the nature of the inflammatory infiltrate: (1) lymphocytic, (2) neutrophilic, and (3) mixed primary cicatricial alopecia [52, 54, 58,95]. The hair follicle is the prime target of the inflammatory process; the mid-portion of the hair follicle with the sebaceous glands and the stem cells (bulge area) are especially affected. Finally, the hair follicle loses its ability to regenerate and is replaced by scar tissue. Lymphocytic primary cicatricial alopecias include chronic cutaneous lupus erythematosus, lichen planopilaris (LPP) with its subgroups, classic pseudopelade of Brocq (PPB), central centrifugal cicatricial alopecia (CCCA), alopecia mucinosa, and keratosis follicularis spinulosa decalvans. Neutrophilic primary cicatricial alopecia comprises folliculitis decalvans and dissecting folliculitis (perifolliculitis abscedens et suffodiens). Folliculitis (acne) keloidalis, folliculitis (acne) necrotica, and erosive pustular dermatosis are classified as mixed cicatricial alopecia.

The most common primary cicatricial alopecias in older patients are LPP and PPB with a typical age of onset around 50 years [14]. Alopecia mucinosa can express a follicular form of mycosis fungoides or can occur without any underlying malignant pathology. The age of onset is usually 50 years or older. Alopecia mucinosa is an extremely rare condition that presents with indurated, well-margined erythematosus patches of scarring or nonscarring alopecia that can be accompanied by diffuse hair loss and alopecia of the eyebrows [26, 86]. Therefore, this condition can be mistaken for alopecia areata or other cicatricial hair loss conditions. A complete work-up to rule out an underlying malignancy such as mycosis fungoides and Sézary syndrome is necessary. No effective standard therapy is available. Oral corticosteroids [60], minocycline [3], and isotretoin [4] have been shown to be effective. Topical and intralesional corticosteroids [23], dapsone [30], indomethacin [43], and light therapy (von [87]) have also been used with variable outcome [58].

Discoid lupus erythematosus, folliculitis decalvans, dissecting folliculitis, acne keloidalis, and erosive pustular dermatosis can theoretically start at any age, but typically occur in young or middle-aged adults. Keratosis follicularis spinulosa decalvans is an inherited X-linked keratinizing disorder that usually develops during adolescence.

18.6.2.1 Lichen Planopilaris

LPP presents in three different forms: (1) classic LPP, (2) frontal fibrosing alopecia (FFA), and (3) Graham-Little syndrome. Classic LPP more commonly presents in middle-aged to elderly women than in men, while FFA and Graham-Little syndrome almost exclusively occurs in postmenopausal female patients. LPP lesions are characterized by atrophic patches with follicular hyperkeratosis and perifollicular erythema. In classic LPP, the lesions are mostly located in the crown area and may present as one expanding patch or in a more reticulated pattern [71]. FFA is characterized by a band-like frontal, in some cases, circumferential recession of the hair line caused by scarring alopecia [77]. Figure 18.5 shows a 51-year-old female patient with FFA, presenting with a band-like cicatricial alopecia of the frontal hair line and nonscarring alopecia of the eyebrows. Graham-Little syndrome presents with lesions of classic LPP, nonscarring alopecia of axillary and pubic hair and eyebrows, as well as keratosis pilaris on the trunk and extremities [7].

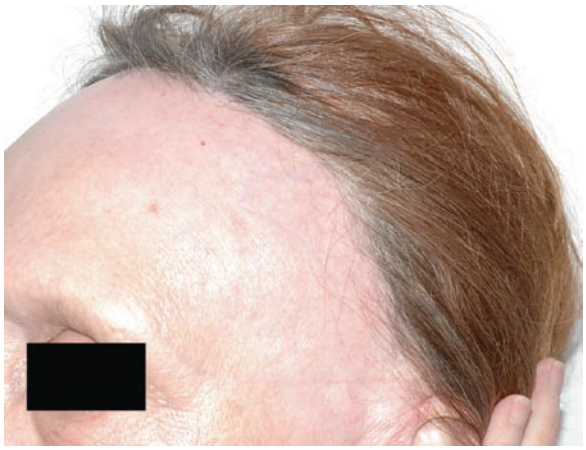

Fig. 18.5 Patient with frontal fibrosing alopecia (FFA), presenting with a band-like cicatricial alopecia of the frontal hair line and nonscarring alopecia of the eyebrows

18.6.2.2 Pseudopelade of Brocq and Central Centrifugal Cicatricial Alopecia

Classic PPB mainly affects women between 30 and 50 years. It typically presents with skin-colored atrophic, alopecic patches with irregular margins; the patches are often interconnected and form a more reticulated pattern. Unlike DLE and LPP, follicular hyperkeratosis and perifollicular or diffuse erythema is mostly absent; however, clinical overlap is frequently seen [31] (Fig. 18.6). CCCA is very similar to PPB. It primarily affects middle-aged black women and starts with one patch in the crown area that slowly expands centrifugally. It remains unclear if chemical processing, heat, traction, or other traumas contribute to the development of this condition [52, 77].

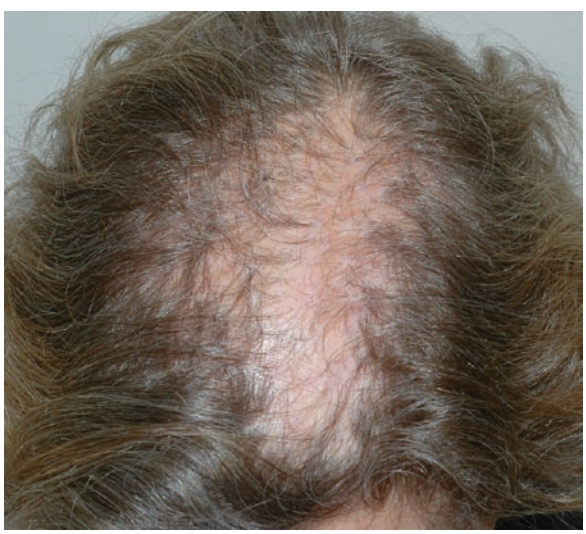

Fig. 18.6 Seventy-three-year-old female patient with pseudopelade of Brocq (PPB)

18.6.2.3 Treatment Options

Cicatricial alopecias are highly distressing for the patient and in general difficult to treat. The patient should understand that the treatment goal is the arrest of the condition and that regrowth cannot be expected. Evidence-based treatment options for any kind of primary cicatricial alopecia are lacking. Literature on effective treatments is meager, especially for the treatment of older patients. Therapeutic options for cicatricial alopecias include topical and intralesional corticosteroids; for more severe or rapidly progressing cases, oral hyrdoxychloroquine, antibiotics, retinoids, corticosteroids, or other immunosuppressants are available as therapies.

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Possibilities and Limitations for Reversal of Age-Related Pigment Loss

19

Ralph M. Trüeb

Core Messages

› The study of hair aging focuses on the aesthetic problem of aging hair and its management, and on the biological problem of hair aging, in terms of microscopic, biochemical, and molecular changes. With today's increasing life-expectation, the desire to look youthful plays a bigger role than ever. The cosmetic industry has become aware of this, and aims to deliver active products that are directed toward meeting this consumer demand. In the absence of another way to reverse hair graying, so far, hair colorants have remained the mainstay of covering lost hair color. Nevertheless, new insights into the biology of the aging hair follicle could open new strategies for prevention or reversal of the graying process. Prominent among these is a focus on the "free radical theory of graying," and an in depth exploration of the maximal potentiality of hair follicle melanocyte populations, their protection and activation.

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> A man from Wan Hsien, in the province of Szechuan, born in the last reigning year of the emperor Ch'ien Lung (1796), worked as secretary to the military office of the Jangtse-region during the final reigning years of the succeeding emperor. Following his retirement, he traveled to Tibet in search of medicinal herbs, and wasn't seen until he was believed to be missing. In autumn 1931 though, he returned in the age of 135 years to his home district, where he was recognized by old townsfolk as someone they had encountered in young days. In spite of his gray hair, he didn't seem older than 50 years, and actually had hardly changed.
>
> Out of the Ta Kung Pao, dated 1939 [6]

The study of hair aging focuses on two main streams of interest: On the one hand, the *esthetic problem* of aging hair and its management, in other words everything that happens outside the skin; on the other hand, the *biological problem* of aging hair, in terms of microscopic, biochemical (hormonal, enzymatic), and molecular changes, in other words the "secret life" of the hair follicle in the depth of the skin.

Hair length, color, and style play an important role in people's physical appearance and self-perception. Civilized mankind's ancient preoccupation with hair is heightened as today's increasing life-expectancy fuels the desire to preserve youthfulness. Hair is not only intended to invoke male recognition of feminine appeal and desirability, but it has also become a predicate upon which social success and career opportunities are based. This attention reflects a hair care market that is a multibillion dollar enterprise. The development of safe and effective means for the treatment of age-related hair changes indicate strategies for maintenance of healthy and beautiful hair in the young and old [47–49].

Finally, basic scientists interested in the biology of hair growth and pigmentation have exposed the hair follicle as a highly accessible and unique model that offers important opportunities also to the gerontologist

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for the study of aging. Its complex multicell type interaction system involving epithelium, mesenchyme, and neuroectoderm, and its unique cyclical activity of growth, regression, rest, and regrowth provides the investigator with a range of stem, differentiating, mitotic and postmitotic terminally differentiated cells, including cells with variable susceptibility to apoptosis, for study. A number of intrinsic and extrinsic modulating factors for hair growth and pigmentation have been identified and are being further tested in vitro [46].

19.1 Natural Hair Graying

Hair graying (canities) is a natural age-associated feature. The hair graying trait correlates closely with chronological aging and occurs to varying degrees in all individuals. While the normal incidence of hair graying is 34 ± 9.6 years in Caucasians and 43.9 ± 10.3 years in Africans, it has been described that, by 50 years of age, 50% of people have 50% gray hair. This graying incidence appears irrespective of sex and hair color. In men graying usually begins at the temples and in the sideburns. Women will usually start around the perimeter of the hairline. Gradually, the gray works its way back through the top, sides, and back of the hair.

Although graying is understood as a loss of pigment in the shaft, its cellular and molecular origins are incompletely understood [46]. Theories for the gradual loss of pigmentation include exhaustion of enzymes involved in melanogenesis, impaired DNA repair, loss of telomerase, antioxidant mechanisms, and anti-apoptotic signals [2, 27, 40, 42, 51].

19.2 Premature Hair Graying

The rate at which an individual turns gray depends on genetics. It is not uncommon to observe kinships with marked early graying throughout. Hair is said to gray prematurely if it occurs before the age of 20 in Caucasians and before 30 in Africans. While premature canities more commonly appear without underlying pathology, presumably inherited in an autosomal dominant manner (familial premature graying), it has been linked to a similar cluster of autoimmune disorders observed in association with vitiligo, i.e., pernicious anemia and autoimmune thyroid disease, and several rare premature aging syndromes, such as Hutchinson-Gilford and Werner's syndrome. In dystrophia myotonica of Curshmann-Steinert the onset of gray hair may precede the myotonia and muscle wasting. In Böök's syndrome, premature graying is associated with premolar hypodontia and palmoplantar hyperhidrosis, transmitted as an autosomal dominant trait [8].

Reports linking cigarette smoking with premature gray hair [23] have drawn on the one hand the attention to the role of oxidative stress on hair growth and pigmentation [50], on the other to canities as a marker for the general health status. A possible explanation of the observation may be that smoking-related diseases increase aging in general, including pigmentation. However, more direct effects, e.g., via smoke genotoxin-induced apoptosis, may also be involved. Whether canities, premature or otherwise, is a predictor or risk marker for disease remains controversial, mainly due to poor epidemiologic study design. Moreover, if it exists at all, it is more likely to reflect associated genetic effects rather than direct linkage [44].

Canities subita is the term used for the rapid onset or "overnight" graying of hair [14, 16]. Although the actual incidence is rare, this stigmatizing phenomenon has captured storytellers' imagination like few other afflictions and occurs to protagonists as a sign of grave sorrow. History records that the hair of the unhappy Queen Marie Antoinette of France (1755–1793) allegedly turned white the night before her last walk at the age of 38 to the guillotine during the French Revolution, giving the phenomenon its name "Marie Antoinette syndrome" [24]. Also the hair of the English martyr Sir Thomas More (1478–1535) turned white overnight in the Tower of London before his execution. More modern accounts refer to the turning white of hair in survivors of bomb attacks during World War II. In 1957, an American dermatologist witnessed a 63-year-old man's hair turn white over several weeks after the subject had fallen down some stairs. The patient noticed loss of hair but no bald patches and 17 months later had extensive vitiligo [13]. Today, the phenomenon is interpreted as an acute episode of alopecia areata, in which the very sudden "overnight" graying is caused by the preferential loss of pigmented hair in this supposedly immune-mediated disorder [46]. This observation has led some experts to hypothesize that

the autoimmune target in alopecia areata may be related to the melanin pigment system [30].

19.3 Graying Due to Nutritional Deficiencies

The quantity and quality of hair are closely related the nutritional state of an individual. In instances of protein and calorie malnutrition, deficiency of specific essential amino acids, trace elements, and/or vitamins, both hair growth and pigmentation may be impaired. The effects of nutrition on hair growth have been recognized from observations in rare inborn errors of metabolism, more so in deficiency disorders, and finally from supplementation studies.

Inborn errors of metabolism with hypopigmentation of hair include: phenylketonuria, homocystinuria, Hartnup disease, and methionin malabsorption syndrome ("oast-house" disease), and nutritional deficiencies known to affect the pigmentation of hair include: protein-calorie malnutrition, and deficiencies of vitamin B12, essential fatty acids, zinc, copper, or selenium.

Vitamin and nutritional deficiencies are common in the elderly population. As many as 50% have a vitamin and mineral intake less than the recommended dietary allowance, and as many as 30% of the elderly population have subnormal levels of vitamins and minerals [18]. A physiologic decline in food intake, regardless of illness, and is often referred to as "anorexia of aging." It probably involves alterations in neurotransmitters and hormones that affect the central feeding drive and the peripheral satiation system. Ultimately, underlying pathology and medical treatment can cause anorexia and malnutrition. Problems with dentition and disorders of the gastrointestinal system are related to poor intake and malabsorption of nutrients. Many diseases (i.e., thyroid, cardiovascular, and pulmonary disease) lead to an increased metabolic demand, and at the same time decreased appetite and caloric intake. According to a survey done in Canada in 1998 [10], 30% of the population reported suffering from more than one chronic health problem, and the percentage increased with age. In the United States, the prevalence of multimorbidity among those 65 and older has been estimated at 65% [56]. Finally, drugs may affect nutritional status through side effects (i.e., anorexia, nausea, and altered taste perception) and through alteration of nutrient absorption, metabolism, and excretion.

19.4 Graying Induced by Drugs

Some systemic drugs may alter the hair color by interfering directly with the pathway of melanin synthesis, i.e., the antimalarials chloroquine and hydroxychloroquine, in others the mechanism is not known. Chloroquine and hydroxychloroquine interfere with phaeomelanin synthesis, and therefore only affect blonde- and red-haired individuals. After 3–4 months treatment hair becomes increasingly silvery or white, usually patchy at first, and in the region of the temples or eyebrows. The changes are reversible. Pigmentary loss of hair induced by drugs has also been described with (in alphabetical order): bleomycin, etretinate, fluorobutyrophenone, haloperidol, mephenesin, tamoxifen, triparanol, valproat, and verapamil. Etretinate, fluorobutyrophenone, and triparanol interfere with keratinization and cause hypopigmented and sparse hair. The color alteration may be due to impaired incorporation of melanosomes into cortical keratinocytes of the hair shaft or an optical effect due to derangement in the substructure of the hair fiber [36].

19.5 Reversal of Hair Graying

Besides substitution in the case of nutritional deficiencies, discontinuation of a drug putative to be the culprit, and avoidance of cigarette smoking, there is no natural way of preserving dark hair or reversing the process of hair graying.

Instances of scalp hair repigmentation have been reported on the following occasions: postinflammatory [4, 31], after radiation therapy [1], following photosensitive dermatitis [5], associated with adult celiac disease [15], following erosive candidiasis of the scalp [53], in Parkinson's disease treated with carbidopa and bromocryptine [34], after chemotherapy [3], associated with etrenitate therapy [54], therapy with cyclosporine A [32, 38], in porphyria cutanea tarda [41], in a case of disseminated granuloma annulare treated with defibrotide [37], in a hepatitis C patient treated with interferon and ribavirin [19], after thyroid

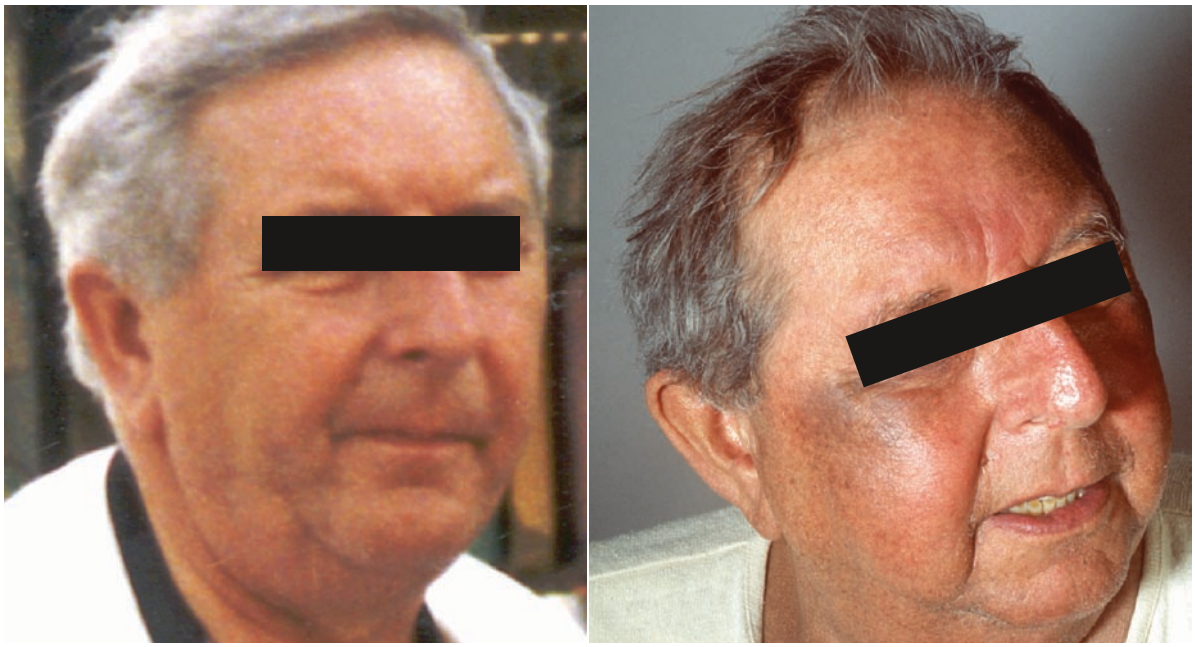

Fig 19.1 Case of spontaneous repigmentation of hair

hormone treatment [33], and after systemic corticosteroids for bullous pemphigoid [21].

The reports of spontaneous repigmentation of white hair (Fig. 19.1) are very scant in the medical literature [9, 29, 45], though this phenomenon may not be as rare as assumed. In fact, it is not too uncommon to see spontaneous repigmentation along the same individual hair shaft in early canities. Moreover, melanocytes taken from gray and white hair follicles can be induced to pigment in vitro [46]. The most dramatic cases of return of normal hair color from gray are probably examples of pigmented hair regrowth following alopecia areata. The reported repigmentation of gray hair in association with Addison's disease has also been connected to a mechanism similar to that in alopecia areata or vitiligo, in view of the known association between these diseases [11]. Alternatively, it may be explained through the effect of elevated levels of MSH, which also applies to darkening of skin and hair in Nelson's syndrome and ectopic ACTH syndrome [36]. Since the stimulation of pigment formation may also affect the hair, a conspicuous darkening of the hair should suggest the possibility of these disorders [60].

Temporary hair darkening has been reported after ingestion of large doses of *p*-aminobenzoic acid (PABA) [43, 59]: Sieve [43] gave 100 mg three times daily to 460 gray-haired individuals and noted a response in 82%. Darkening was obvious with 2–4 months of starting treatment. The hairs turned gray again 2–4 weeks after stopping therapy. The mechanism of action has remained unclear. Major drawback are gastrointestinal side effects. For this reason, PABA is usually incorporated in smaller doses in commercially available oral dietary supplements for hair growth and color.

Darkening of hair has also been observed in the course of treatment of androgenetic alopecia with the topical hair growth promoting agent minoxidil. The mode of action is probably prolonged anagen and follicular enlargement, enhancing normal melanogenesis [12].

19.6 Hair Colorants

In the absence of another way to reliably reverse hair graying, hair colorants have remained the mainstay of recovering lost hair color, reaching back as far as to the Ancient Egyptians who colored their hair with henna and indigo, and the Ancient Romans who used lead combs dipped in vinegar. Henna, obtained form the plant *Lawsonia alba*, is a naturally occurring hair colorant still frequently used today. Although the color can add red highlights to hair, occasionally on gray hair it may come out looking orange.

There are several choices open to a person with gray hair: To apply blond streaks to some of the hair, a procedure called highlighting. To color only the gray, especially in the beginning when the gray in men affects only the temples, or the perimeter in women. To color about half the hair by wrapping it with a lighter shade producing a natural look. Finally, to color the entire head of hair, usually going two shades lighter than a person's natural color to prevent a harsh look. The following major types of synthetic hair colorants are currently used: temporary (textile dyes), natural coloring (henna), semipermanent (low molecular weight direct dyes), and permanent (aromatic amines).

Temporary hair colorants consist of large complex organic structures that do not penetrate the cuticle. The colors are not intense but are capable of covering gray hair in a subtle way. This may be a good way for an individual to experiment with the coloring idea. The colorant washes out with the next shampoo.

Semipermanent colorants consist of small molecules that penetrate the cuticle. These compounds color gray hair very nicely, are easily applied in a lotion or foam at home, and last for six to ten shampoos.

Permanent hair dye is the most frequently used hair colorant. In permanent hair coloring the formation of colored molecules from their precursors occurs inside the hair fibers as a result of oxidation by hydrogen peroxide. The advantage of permanent color is that the color withstands normal hair washing. Because new growth comes out, the roots need to be touched up. Such products are used in a very gratifying manner and safely by millions of individuals worldwide. Besides a cosmetic benefit, hair dyes have been shown to also have a photoprotective effect on the hair fiber [28].

There have been studies that raised the possibility that long-term usage of permanent hair dyes (particularly black dyes) may be associated with an increased risk of developing certain cancers**.** However, taken together the evidence is insufficient to state with certainty whether there is a link between using hair dye and cancer [7, 20, 22, 35].

19.7 Future Developments

Possibilities for prevention or reversal of senile graying of human hair have been the subject of intense research since ancient times, but not until recently has some progress been made in the understanding of the underlying biology of hair pigmentation and its derangements, respectively.

Since reactive oxygen species have been implicated in hair follicle melanocyte apoptosis and DNA damage, and under in vitro conditions Met oxidation of tyrosinase could be shown to be prevented by L-methionine, it will be interesting whether L-methionine could be useful for intervention or reversal of the hair graying process [57].

There is also an increasing interest in the hair follicular route for delivery of active compounds affecting the hair. Current research activities focus on topical liposome targeting for melanins, genes, and proteins selectively to hair follicles for therapeutic and cosmetic modification of hair [17].

Another line of research in the quest of new treatments for loss of pigmentation is tissue engineering with cells of hair follicular origin with stem cell properties [52, 58]. Advances in the identification and characterization of stem cell populations [25, 26] has led to substantial interest in understanding the precise triggers that would operate to induce activation of quiescent stem cells. Melanocyte stem cells that reside in the bulge region of murine hair follicles are characterized by reduced expression of the microphthalmia-associated transcription factor (Mitf) and its target genes implicated in differentiation. As Mitf is implicated in control of proliferation, Saha et al. [39] explored the possibility that inducing Mitf expression via lipid-mediated activation of the p38 stress-signaling pathway may represent a repigmentation strategy. They isolated from placental extract a C18:0 sphingolipid able to induce Mitf and tyrosinase expression via activation of the p38 stress-signaling pathway. Strikingly, in age-onset gray-haired C57BL/6J mice that exhibit decaying Mitf expression, topical application of placental sphingolipid led to increased Mitf in follicular melanocytes and fresh dense black hair growth.

19.8 Concluding Remarks

Graying as a visible manifestation of the aging process has attracted the attention of the sciences as early as can be traced in written documents. It is not until recently though that the biology of hair pigmentation and graying have begun to be elucidated. Prominent

among these is a focus on the aging hair follicle, the "free radical theory of graying" [2], and an in-depth exploration of the maximal potentiality of hair follicle melanocyte populations [25, 26]. Prevention and reversal of the hair graying process may become feasible through the precise identification and efficient delivery of appropriate protective and/or trigger factors that would operate to protect melanocytes from oxidative stress [55, 57] or induce the activation of quiescent stem cells [39, 52, 58], respectively.

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Evidence-Based Hair Transplantation

20

Andreas M. Finner

Core Messages

- Hair transplantation is a permanent treatment option for androgenetic alopecia in suitable male and female patients.
- The best long-term outcome is achieved in medically or spontaneously stabilized hair loss. In these cases, the redistributed hair increases density and scalp coverage.
- Realistic expectations, a good donor-to-recipient area ratio, careful personal planning, and a skillful surgical team are essential.
- Follicular unit transplantation is the standard, involving dense placement of very large numbers of natural bundles of 1–4 hairs into recipient sites of appropriate size, angle, distribution, and direction.
- These transplanted hairs are virtually undetectable from the original hair. Thus, progressive alopecia is no longer a contraindication per se, if sufficient donor hair is available.
- The grafts are harvested with local anesthesia through strip excision with trichophytic closure to keep the scar minimal and/or by follicular unit extraction. Both harvesting methods have individual indications.
- Stereomicroscopic dissection with backlighting and the use of loupes for recipient site creation greatly reduce transection rates and temporary effluvium of preexisting hair.
- The hairline is created based on individual characteristics with micro- and macroirregularities, including a frontal transition zone of single hair units.
- More quantitative evidence from controlled trials is necessary to evaluate special techniques for each component of the procedure.

20.1 Introduction

Hair restoration surgery is a permanent treatment for androgenetic alopecia (AGA) in suitable candidates. It includes hair transplantation (HT) and, only in a few skilful hands, HT combined with scalp reduction, the use of a scalp extender, or flap surgery.

HT is based on the principle of donor dominance of androgen-independent follicles retaining their properties when they are transplanted into androgen-dependent areas. The donor supply is limited by the area of the "safe zone," scalp elasticity, and the density of donor hair. During the surgery, hairs are redistributed over the scalp to optically restore coverage.

Worldwide, HT is performed several hundred thousand times yearly.

However, quantitative evidence in hair restoration surgery based on randomized controlled trials is insufficient. This may be due to many reasons, such as high variation in techniques, multiple steps in the

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surgical process, problems in measuring hair growth, and difficult patient recruitment.

Modern HT is a complex procedure, which requires careful planning and a well-trained, skilful team of the surgeon and several assistants. This greatly determines the success and aesthetic result of the surgery.

With recent advancements in the technique, larger numbers (up to 3,000) of smaller grafts are moved per session, and results have thus become very natural [5, 24].

Follicular unit transplantation (FUT) has become the standard technique in HT [3, 4, 19, 22]. These natural bundles of 1–4 hairs require smaller recipient sites and can be placed much denser.

Using larger multi-FU grafts or pairing FU into one recipient site is only recommended in the center of the recipient area mixed with pure FU sites. They increase fullness and coverage but should only be used if there is a good recipient/donor ratio or with good hair characteristics such as lighter, curly, or finer hair.

With the proper use of FUT and an esthetically correct placement and distribution, grafts can stand on their own even if preexisting hair is lost. Thus, progressive AGA is no longer a contraindication for HT per se.

However, in medically or spontaneously stabilized AGA, the long-term result is better because preexisting hair is no longer lost and the density stays the same. Otherwise, follow-up surgery may become necessary. But even this cannot numerically replace all lost hairs. This should be taken into account before starting any surgery.

Theoretically, the numerical threshold of 50% original density above which hair loss becomes invisible should be aimed for in crucial areas. However, this will not be possible with one procedure of HT in a completely bald area. Often, a cosmetically satisfying density can be achieved with proper and elegant graft placement in one session of surgery, especially in patients with good hair characteristics and longer hair styles.

20.2 Indications and Planning

Suitable candidates for HT are those with reasonable expectations, a donor supply that is adequate to cosmetically improve the recipient area coverage and those without contraindications for surgery. In thicker, blond, gray, and fair hair, better results can be achieved with less grafts.

A personal consultation between the surgeon and the patient at least several days prior to surgery should be standard, which is also necessary to exclude dysmorphophobia and other contraindications for surgery. A realistic result, the hairline and graft number and distribution as well as the number of procedures and the difficulty of predicting the exact outcome should be discussed with the patient at length.

An exact diagnosis should be made including the stage and progression of AGA. Other forms of hair loss – telogen effluvium, alopecia areata, or active cicatricial alopecia – and concomitant scalp conditions such as actinic keratosis should be excluded.

Obtaining a family history and using old photos may be helpful to estimate the activity of the condition.

The examination also includes the assessment of hair quality and the donor and recipient areas. The use of digital imaging methods to assess the donor area and the activity of the condition in the affected areas may improve this planning process.

Standardized global photos should always be taken before therapy and on follow-up appointments. This increases compliance and facilitates the assessment of activity.

The most dramatic change in cosmetic appearance is achieved in patients with stages Hamilton–Norwood V and VI and in patients with anterior accentuation of balding (subtypes IIIa and IVa). The severity of AGA can be improved by several stages, depending on the number and extent of procedures.

Especially in younger patients with progressive alopecia or in patients with limited donor hair, it may be advised to leave out the vertex area during the first procedure(s) and concentrate on the fronto-parietal scalp and hairline, which frames the face and restores its proportions.

If the patient is satisfied with the fronto-parietal result, no further hair loss of preexisting hair is expected in that area, and when there is remaining donor hair, the vertex may be transplanted. However, if the vertex alopecia is still progressing, the surgeon has to take into account that additional procedures are required.

Patients stage III and IV can very well undergo HT. But, these patients should be informed that additional procedures may become necessary if the preexisting

hair is lost over time. This is only possible with sufficient donor hair and financial resources.

Such an early, sequential approach allows a more gradual thickening of the recipient area, which is less abrupt and noticable to others. It is also possible and advisable to distribute grafts in between thinning preexisting hairs using magnification and bright illumination and thus account for future hair loss [7].

The preexisting hair can be used as an orientation for the direction, angle, and distribution of the grafts.

If AGA stage III or IV has stabilized spontaneously or with medical therapy and this has been documented over at least 12 months, the surgery may concentrate on the more frontal areas of the scalp such as the bitemporal recession. However, medical therapy should not influence the planning and design of the procedure, mainly because of compliance problems and because AGA may follow an intermittent, episodic course. The surgical plan should always account for possible progression.

In practice, this means a sufficient donor reserve should be left for follow-up surgery, the hairline should be designed conservatively, and grafts should also be placed in-between hairs, which are likely to miniaturize.

In patients with stage VII, the possible result is limited by a high recipient-to-donor-area ratio. If the donor supply is very good, transplants can be distributed evenly in a low density. Alternatively, the surgery has to concentrate on the frontoparietal area or a frontal forelock is created [13]. If the patient has higher expectations, he should opt for a hairpiece.

Patients with early AGA Norwood–Hamilton stage II are not good candidates and should go on medical therapy first if there are hints for activity in the physical or digital examination or a clearly positive family history. Again, photodocumentation is crucial.

In women, HT is indicated in male patterned thinning, in Ludwig stage II and the frontal accentuation type of AGA (Christmas tree pattern). With the use of smaller grafts and more careful recipient site creation, very satisfying results can be achieved [17, 25]. A dense donor area is a prerequisite. Most women require two or more procedures and often the recipient area has to be limited to the cosmetically most important regions, such as the midline or preferred side of parting. Because grafts are placed between preexisting hairs, temporary partial telogen effluvium (shock loss) is more common in women. It may be lessened by the use of minoxidil topical solution [2].

It must be emphasized that for optimal long-term results, surgical treatment should be combined with medical treatment in patients stages up to VI, who still have preexisting hair in androgen-dependent areas and progressive AGA [6]. This is especially important for the midscalp and vertex area where medical therapy has the highest efficacy. A study comparing patients who underwent surgery with and without finasteride 1 mg treatment showed better results in the combination group [11]. Minoxidil may also improve and accelerate results of the surgery [2].

20.3 Techniques

HT is an outpatient procedure and may take 3–8 h, depending on various factors, especially the number of grafts. Tumescence anesthesia is used for the donor and recipient areas, sometimes combined with nerve blocks [15].

20.3.1 Donor Hair Harvesting and Follicular Unit Dissection

The most commonly used technique in the donor area is strip harvesting. It allows for a relatively fast removal of large numbers of hairs leaving a fine line as a scar. Several techniques are used to assess donor density and estimate the required strip size [16]. Digital imaging techniques are helpful to assess FU density and composition and strip size (personal observation). It is advisable to use a long but narrow strip to improve the resulting scar.

The elliptic strip is excised by double-bladed knives or freehand with a scalpel [9], alternatively with blunt spreading instruments after a superficial incision. The scar is covered by adjacent hairs, which should be at least 2 cm in length. Thus, the patient can no longer have a short (“buzz”) hair cut after the procedure.

Special wound closure techniques such as the “‘trichophytic closure” have been shown to produce a minimal, less visible scar [10].

The strip is dissected into follicular units under magnification, preferably a stereomicroscope with backlight. When well-trained assistants are available,

this leads to much lower transection rates and smaller grafts with less interfollicular tissue. FUT allows for smaller recipient sites (i.e., slits or holes) and higher densities. It is not clear yet how much the grafts should be trimmed. Too skinny grafts are certainly more vulnerable, even with very careful graft handling. Dissection quality and speed rely on the skills of the staff and greatly determine the result of the procedure, because the grafts must always stay wet and cool and should be out of the body as short as possible. Special storage solutions are used, but their effect on graft survival and quality when compared with normal saline requires further evaluation.

A recent technique involves separately harvesting individual follicular units using small punches of 1 mm or of lesser diameter [14]. It is called follicular unit extraction (FUE). Advantages include less extensive surgery, avoiding a long scar and less staff for microscopic dissection. Those patients with a small recipient area, low donor density, or minimal scalp laxity may be candidates.

Disadvantages are lower graft numbers, less fine hair for the frontal hairline, several sessions, multiple fine pin-point scars, the shaving of the donor area prior to harvesting, more time and physical effort for the surgeon, and most importantly, a higher transection rate despite the latest use of blunt punches after a superficial incision. A preoperative test to assess individual FUE feasibility is advisable.

Today, hair surgeons should be able to perform both harvesting methods. A combination of initial strip surgery and subsequent FUE may increase the graft yield. Further studies are needed to define the role of FUE for certain patient subgroups.

20.3.2 Recipient Site Creation and Density

Recipient sites are made with solid core needles or spear-like microscalpels of various sizes and with different tips. The size, direction, and angulation of the incision should be adjusted to graft size and location [1, 23]. Using loupes and assessing preexisting hair may greatly facilitate orientation. Most surgeons start with an acute frontal angle, which increases when going backward on the scalp. Varying the direction of the hair is proposed by many authors (coronal vs. sagittal, or oblique), as well as changing the orientation of the blade (parallel vs. perpendicular). However, sagittal excisions cause less damage to preexisting hair.

The density of the grafts depends on the area, graft size, and available number of grafts [12, 20, 21]. Several small case series indicate a lower graft survival above densities of 40 FU/cm^2 placed at once. Therefore, the so-called "dense packing" cannot be recommended routinely, especially if large graft numbers in mega-sessions additionally impair vascularization.

In general, large mega-sessions with more than 2,000 grafts should only be performed by a big, skilled, and fast team and with ideal patient characteristics (large donor supply, easy placing, good healing, etc.).

20.3.3 Hairline Design and Graft Placing

Creating the hairline and making the hairline incisions are the most critical and demanding parts of the procedure. The hairline should be designed according to the patient's face and following several aesthetic rules [18]. It should never be too low and is placed horizontally to the ground or slightly upward toward the fronto-temporal apex.

A hairline with macro- and microirregularities with a transition of fine, staggered single hairs toward thicker hair and 2-hair-FUs in a denser packed zone immediately to the posterior gives best results.

Restoring the temporal triangles greatly improves the esthetic appearance [8]. Sometimes, the lateral humps have to be recreated as well.

The grafts can be spread out evenly over the entire recipient area or be packed denser on the frontal and mid scalp depending on donor supply, possible subsequent sessions, and patient demands. In the latter approach, a transition zone with smaller grafts should also be created toward the vertex. It is also advisable to place grafts in between miniaturizing hairs, which is done with the use of loupes [7].

Gentle graft placement is usually done with jewelers' forceps. Bleeding and popping of grafts may complicate this. This step also requires very good manual

skills. If the epidermis has not been removed from the grafts, they should be placed above the skin level; otherwise they are placed flush.

20.3.4 Postoperative Care and Results

The patient should be warned about risks of the surgery, the time line before regrowth (6 months or more), and the initial loss of transplanted hair and a portion of pre-existing hair in the recipient area (shock loss).

It has not been established whether bandages and moist dressings with or without special components provide an advantage over no dressing and blow-drying of the scalp after surgery. Postoperative care involves gentle shampooing from the second or third day after surgery.

Swelling of the forehead is common between days 2 and 7. Though cooling is routinely recommended, not all surgeons use steroids orally, intramuscularly, or in the tumescence preparation for prevention.

Usually, patients can resume their working schedule and other activities 7 days after the surgery.

Infection is very rare. Perioperative antibiotics are frequently used.

Pain and dysesthesia are common in the donor area immediately postoperatively and rarely for a prolonged time. This is treated with pain killers. The suture is removed 10–14 days postoperatively.

The final result of the procedure cannot be expected before 9 months postsurgery, and 6 months is the earliest time for potential subsequent procedures. Although more studies are needed, a graft survival above 90% can certainly be achieved when all the components of the procedure are carefully performed.

20.4 Conclusion and Outlook

In conclusion, hair restoration surgery today can produce excellent long-term results in suitable candidates with an experienced team, especially FUT with the use of micro-instrumentation in combination with medical therapy. More high-quality studies with larger patient numbers are necessary to determine the optimal settings and technical components of the procedure.

In the future, automating parts of the procedure could reduce costs and the human factor. Hair multiplication through tissue engineering techniques would provide unlimited donor supply.

However, hair restoration is a very individual procedure and always has an artistic component depending on the aesthetic and creative skills of the hair surgeon.

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Future Directions: Gene Polymorphism Diagnostics Relevant to Hair

21

Justine A. Ellis

Core Messages

- Hair disorders related to ageing, such as androgenetic alopecia and hair graying, are controlled, at least in part, by genes.
- The aetiology of such disorders is complex, with many interacting genes likely to contribute to the risk. The action of these genes may also be influenced by the environment.
- At present, we know very little about these complex genetic risk profiles; for androgenetic alopecia, only one gene has been identified.
- This lack of understanding is a barrier to the translation of genetic research findings into patient care.
- However, as more genes are identified, genetic research has the potential to predict risk and take preventive action, to accurately diagnose hair conditions, to develop targeted treatment strategies and to apply personalised treatment strategies based on an individual's underlying genetic make-up.

21.1 Introduction

It is well established that hair disorders, particularly those related to ageing such as common baldness [8, 20] and hair graying [31], are complex in their aetiology. The occurrence and age at onset of male and female pattern hair loss and the age at onset of hair graying appear to be heritable characteristics in humans. In male pattern baldness, or androgenetic alopecia, twin studies have shown that the development of hair loss is almost entirely determined by genetic predisposition [26]. But, the wide distribution of age at onset and severity in the population strongly suggest that this condition is not controlled by just one gene, rather it is polygenic [8, 20]. Like many polygenic human disorders, the heritability of androgenetic alopecia will be dependent on a complex interplay between a number of genes throughout the genome, each contributing variably to the risk of hair loss across the lifetime. Additionally, there may be other genomic factors that involve other mechanisms of genomic control apart from traditional sequence variation in known genes.

The remarkable advances in genetic and genomic discovery over the past few years have opened up new opportunities for geneticists to begin to uncover the genetic architecture of complex polygenic diseases and phenotypes, but this area remains in its infancy [24]. For androgenetic alopecia, one gene, the androgen receptor (*AR*) on Chromosome X, has been confirmed as harbouring genetic variation that contributes to the risk of hair loss [9, 15, 16, 21]. Since this discovery, eager but perhaps unscrupulous organisations have begun to market personalised tests designed to provide consumers with a risk estimate for the development of androgenetic alopecia based on *AR* genotype. As will be further explained in this chapter, only a relatively small portion of the heritability has been explained by variation in *AR*, and thus this is a very premature outcome of the genetic research. What is needed is a more thorough understanding of the overall genetic risk profile, which could involve tens, or even hundreds, of genes that may differ in their combinations between

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affected individuals, the way in which they interact and the ways in which the genome controls them, before we can fully appreciate the complexity of this hair condition. However, when we do understand the complexity, the opportunities for using this knowledge to define new therapeutic targets and preventive strategies and to tailor such therapies and strategies to individual risk profiles (the so-called "pharmacogenomics" or "personalised medicine") are exciting and enticing.

21.2 Complex Human Disorders

21.2.1 Single Gene Disorders

The father of modern genetics, Gregor Mendel, first described the basic laws of genetic inheritance based on plant breeding experiments in the nineteenth century. The characteristics of Mendel's peas, such as colour and shape, were shown to be inherited in either a dominant (one allele required for expression of phenotype) or recessive (two alleles required for expression) pattern. In the early and mid twentieth century, this knowledge was used to help define the heritability of single gene disorders, now known as Mendelian, or monogenic, disorders, such as phenylketonuria (PKU, autosomal recessive) and Huntington disease (HD, autosomal dominant) [25]. Armed with this knowledge, and advances in genetic technologies, the late twentieth century saw the discovery of a plethora of genes that are responsible for relatively rare diseases determined by a single gene. These genes are generally easy to detect, since all affected individuals within family lineages would have inherited the genetic defect, while unaffected family members would not. The defective gene is necessary, and usually sufficient, to cause the disorder, often at a similar age and with a similar degree of severity. Many of these disorders were later found to have a slightly higher degree of complexity; for example, the actual causative mutation within the gene might differ from family to family (such as in cystic fibrosis where over 1,000 variant alleles have now been discovered in the CFTR gene [6]), and this might dictate distinct phenotypic subsets of the disorder [1]. However, in comparison with more complex disorders, the genetic architecture has been relatively simple to understand and define.

21.2.2 Oligogenic Disorders

The next step up in complexity might be said to be a small subset of disorders that have what has been termed oligogenic inheritance. This pattern of inheritance is characterised by two, or a small number, of genes that contribute in a major way to the development of the disorder. Usually, no singe gene is sufficient to cause the disorder; rather, all are necessary in combination [1, 25]. An example of an oligogenic disorder is a form of retinitis pigmentosa that is caused by the presence of mutations in two separate genes [18]. Around 50 disorders have been postulated to be oligogenic in architecture [1], but there is debate as to whether more complexity in their inheritance is yet to be discovered. It is fair to say that, for this group of disorders, there are at least a small number of genes that make up the bulk of an individual's predisposition to developing them, that is, each of these genes confers relatively high, but not absolute, risk, as is seen for single gene disorders.

21.2.3 Polygenic Disorders

While monogenic and oligogenic disorders are usually determined by one or a few genes that have a strong influence on the phenotype, polygenic traits, often referred to as complex traits, are likely to be determined by a much larger number of genes that confer variable levels of risk. To date, there remains no complex human phenotype, whether the phenotype is considered a disease, disorder or trait, for which the risk profile has been fully determined. Often, complex polygenic traits are not binary in nature, that is the trait does not exist as one state or the other, such as affected or unaffected. For example, human stature is a complex trait, and is measured as a continuous variable that shows a normal distribution across a population [11] (Fig. 21.1). The underlying genetic architecture remains unknown, and even the largest genetic association studies performed to date have found only a handful of genes that contribute to adult stature, which together make up a small percentage of the heritability [35]. It is thought that although height may be up to 80% heritable, each of the genes that contribute to the determination of adult height will account for less than

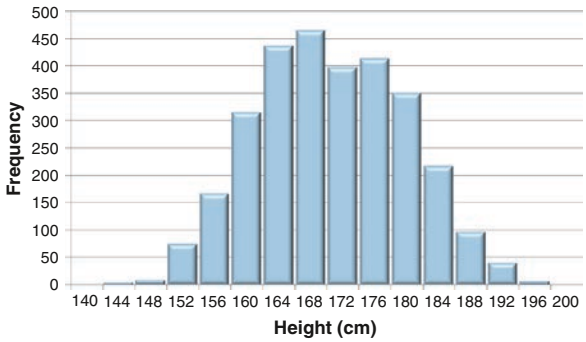

Fig. 21.1 Normal distribution of adult height in participants of the population-based Victorian Family Heart Study. This type of distribution is a hallmark of a complex phenotype. Data courtesy of Professor Stephen Harrap, graph courtesy of Anna Duncan, University of Melbourne

1% of the overall heritability. If this is true, there will be in excess of 80 genes controlling height. Each individual will inherit a distinct set of alleles of these genes, some predispose to growing taller, some to being shorter. In simplistic terms, each allele might add and subtract a centimetre or two from an individual's final height (note that this is indeed simplistic, as will be discussed later) until the maximum height able to be achieved is determined.

However, as is common for the majority of complex traits, genetic sequence variation is not the only contributing factor that determines the trait. Environmental influences will also play a role, and in relation to adult stature, poor nutrition during childhood and adolescence will prevent an individual from reaching their peak height achievable based on their genetic make-up [30]. It is this synergistic interplay between genes and environment that determines a complex phenotype. In fact, two individuals who achieve exactly the same peak adult height are unlikely to do so because they share exactly the same set of gene variants, and have been exposed to exactly the same environmental influences. It is most likely that a different combination of factors has derived the same final adult height. Individual One might have more "tall" genes, but had poorer nutrition during childhood growth phases, while Individual Two might have less "tall" genes, but received optimal nutrition at exactly the right times during childhood.

The complexity of polygenic traits means that the genes determining them are much harder to identify. Simple comparisons of tall and short individuals, as can be done for monogenic disorders, often will not identify predisposing genes, because not all of the tall people will have all of the "tall" genes. In fact, they may have a subset of "short" genes, but these are overcome by good nutrition, or are masked by other genomic control mechanisms that will be discussed subsequently. Conversely, the short individuals may have a subset of "tall" genes.

21.3 Androgenetic Alopecia as a Complex Disorder

We have used adult stature as an example as it is an easy illustration of a complex trait we are all familiar with. However, many of the concepts discussed in relation to height are equally valid for androgenetic alopecia. As previously mentioned, like height, androgenetic alopecia appears to be a highly heritable disorder, though it is thought that few, if any, environmental factors contribute to the risk of developing hair loss [26]. Therefore, this disorder is slightly simpler to study as the effects of genes are unlikely to be masked significantly by the environment in which they are expressed. We know that *AR* is one of the predisposing genes, and studies have demonstrated that a particular single nucleotide change (or single nucleotide polymorphism, SNP) in the first coding exon of *AR* is associated with baldness. That is, one allele of this locus is present in almost all men who develop hair loss, especially if the onset of that hair loss occurs at a younger age (premature baldness) [10]. On the surface, this might seem a useful finding, and one ready to be exploited by pharmaceutical companies for new targeted treatment development. However, this same variant was found in a high percentage of men who showed no evidence of hair loss at age 50 years or older. Additionally, although the associated SNP is found within the coding sequence of the gene, the variant does not change the amino acid of the resulting AR protein that is produced, and therefore has no obvious functional consequence. Therefore, on its own, this discovery though important cannot be used with any accuracy to predict hair loss (at least hair loss by 50 years of age), and because an understanding of the functional difference in *AR* between men with the predisposing allele and men with the non-predisposing allele has not been achieved, it is impossible to use the SNP as a therapeutic target.

It has been estimated that genetic variation in *AR* might account for up to 40% of heritability [16]. Therefore, up to 60% of the heritability remains unexplained. The fact that almost all men with premature baldness have the predisposing *AR* variant suggests that the *AR* variant may be necessary for the development of common hair loss. That is, inheriting a predisposing copy of *AR* seems to be a prerequisite for hair loss. However, because this same predisposing copy of *AR* is seen so commonly in older men with a full head of hair, it is unlikely to be sufficient in itself to cause baldness. The other factors that hold the remaining pieces of the puzzle that determine sufficiency are yet to be elucidated. These factors are likely to include a combination of sequence variation in as yet undiscovered genes. Such variation may not actually affect the coding sequences and therefore the proteins that are produced by these genes, but may affect the regulatory elements within the non-coding sequence that make subtle changes to the expression of genes. They may also include hereditary changes to the genome that are not based on DNA sequence, the so-called epigenetic variation.

21.4 The Complexity of the Human Genome

21.4.1 Genes and the Proteins They Produce

The current estimate for the number of genes in the human genome that encode proteins is somewhere in the vicinity of 25,000 [32]. This is far less than the estimates prior to the Human Genome Project of in excess of 100,000 genes. As our understanding of the complexity of the human genome has grown, we now realise that this smaller set of genes, through a variety of mechanisms of regulatory control and complex interaction, is capable of conferring the amazing degree of variation displayed by the human population. One human individual differs from another by sequence differences at as little as 0.1% of the nucleotide positions that make up our genome [5]. These variants may have a myriad of effects past the traditional, and easiest to understand, variants that affect the amino acid sequence of a protein. The majority of polymorphisms found to cause monogenic disorders like cystic fibrosis do occur in gene coding regions, and make a gross change to the protein that the gene is capable of producing. These polymorphisms are obviously functional in their effect. However, according to publically available SNP databases, only ~3% of SNPs lie in coding regions (dbSNP build 129, http://www.ncbi.nlm.nih.gov/projects/SNP/), the remainder lie in non-coding sequences. As the identification of functional gene coding changes is a relatively rare finding in complex disorders, non-coding variation throughout the genome must be exerting functional effect. In the recent past, many examples of non-coding functional variation have been published [2]. This non-coding variation might lie close to a gene, for example, in a promoter or untranslated region, or it may lie many megabases from, or even on a different chromosome to, the gene upon which it exerts its effect [4].

21.4.2 Regulation of the Genome by Non-Coding DNA

There are many ways in which non-coding DNA can affect the function of a gene, and most are beyond the scope of this chapter. However, the overall concept of the importance of non-coding DNA sequence is important in understanding the complexities of the genome, and the difficulties faced by genetic researchers who are attempting to uncover the genetic basis of complex disorders like androgenetic alopecia.

One way in which non-coding DNA can control gene expression is through regulatory elements [14]. These elements are commonly short stretches of sequence that harbour recognition sequences (binding sites) for proteins that act as transcription factors. An example of a transcription factor is AR itself [23]. When bound to androgen, AR is activated to become a transcription factor that recognises specific binding sites in regulatory regions of genes that respond to the presence of androgen. When the AR–ligand complex binds to these sequences, the androgen-dependent gene is up- or down-regulated, depending on whether the transcription factor binding site acts as a transcription enhancer or suppressor. These transcription factor binding sites may lie a long distance from the gene promoter sequence, and may, for example, fold back to contact with the promoter region to synergistically alter transcription levels [19].

It is important to note that such non-coding regulatory mechanisms are capable of altering gene expression in a subtle way. Unlike the gross physical changes to a protein that might result from gene coding variants, and that might confer high or absolute risk of developing a disorder, non-coding variation has the capacity to subtly alter gene expression, and such alterations might occur in a tissue- or developmental stage-specific manner [4]. In the case of complex polygenic disorders in which each gene variant that makes up the underlying genetic predisposition is likely to contribute minimally to the overall risk, it is reasonable to suggest that non-coding variation of subtle function might be a better candidate than coding variation. Indeed, the results of recent large-scale genome-wide genetic association studies have identified many new variants for a variety of complex disorders, and almost all of these variants have been in non-coding regions, some at a considerable distance from any known genes [24].

21.4.3 Regulation of AR by Non-Coding DNA and Relevance to Androgenetic Alopecia

AR itself is known to be regulated by transcription factors that control its expression. Though no long range regulatory elements have been definitively identified for *AR*, chromosomal translocations that involve truncating the genetic material of chromosome X approximately 134 kb upstream of the *AR* coding sequence itself have been shown to cause partial androgen insensitivity [22], suggesting a down-regulation of *AR* and the existence of regulatory elements far upstream of the gene.

Given the likelihood of the existence of non-coding regulatory elements, and the lack of obvious functionality of the *AR* coding variant associated with androgenetic alopecia, could it be possible that the association of *AR* be accounted for by the non-coding variation? The answer is yes, and this is a very likely explanation. The International HapMap Project [34], a follow-on from the Human Genome Project, has constructed a database of SNPs throughout the genome that travel together from generation to generation. SNPs that are commonly inherited together across generations are said to be in linkage disequilibrium (LD), and groups of SNPs that travel together in this way form a "haplotype." The beauty of the LD data from the HapMap Project is that it is possible to predict haplotype blocks that occur in the population, and rather than genotyping all SNPs in that haplotype block, one or a small number of SNPs that represent that haplotype, the so-called "tag" SNPs, can be genotyped to fully define the genotype of each SNP in the block. Thus, these tag SNPs can act as proxies for other SNPs with which they are in tight LD.

An analysis of the region on chromosome X that contains *AR* demonstrates a lengthy block of LD encompassing the gene coding sequence (including the androgenetic alopecia-associated exon 1 SNP), 900 kb of sequence upstream of the gene and even some sequence of the next gene proximal to *AR* [12]. Thus, the androgenetic alopecia-associated *AR* SNP is acting as a tag SNP, or a proxy, for hundreds of other SNPs in the region. This means that the association that was detected between androgenetic alopecia and *AR* could be explained by the presence of another functional SNP that is travelling together with the exon 1 coding SNPs through the generations, and for which the exon 1 SNP is acting as a proxy. This other functional SNP may exist in the vast length of non-coding regions upstream of *AR*, or even within the next gene upstream. Interestingly, recent studies have demonstrated even stronger associations of androgenetic alopecia with SNPs in the *AR* upstream non-coding regions [16] (Ellis et al., unpublished), and even within the proximal neighbouring gene [28].

Though this is an important knowledge, it helps little at the current time in defining the functional variant in *AR* that is predisposing to androgenetic alopecia. This is because the non-coding DNA remains poorly annotated, and it is very difficult to define with any confidence the important non-coding regulatory sequences. Without this knowledge, we cannot determine which of the hundreds of SNPs that exist in the *AR* haplotype block might exert a functional effect. Large-scale international efforts, such as the ENCODE project [3], aim to determine common characteristics of important non-coding DNA. Other methods such as comparative genomics, where human genome sequences are compared with other species with the hypothesis that important sequences will be conserved through evolution, are also being developed [4]. Until non-coding DNA annotation is well defined, it remains a virtually impossible task to prioritise large numbers

of non-coding SNPs for the examination of function, and thus contribution to complex disorders.

21.4.4 Regulation of Genes by Non-Sequence-Based Mechanisms – Epigenetics

Another striking example of the complexity of gene regulation lies in non-sequence-based variation. Epigenetic mechanisms such as DNA methylation serve to modify the DNA and make it more or less accessible to the machinery required by the genome to effect transcription. Methylation of cytosine bases occurs in CpG islands (that is, in sequences where C and G bases lying side by side are far more common than in the general genome sequence). Such CpG islands often occur in the promoter regions of genes, and when the cytosine bases are methylated, the transcriptional "machinery" is blocked from docking with the promoter sequences, thus blocking transcription of the gene. When CpG islands are unmethylated, the way is free for the machinery to dock and effect transcription. A striking example of this process is in X inactivation, where the promoter region of the majority of genes on one of the two copies of the X chromosome in females is hypermethylated (and thus transcription is prevented), and those on the other X chromosome are not [33]. This provides a means to control gene dosage.

Interestingly, evidence is emerging that methylation is capable of not only turning on and shutting off genes, but may also be capable of more subtle control of expression [36]. For instance, partial demethylation of a gene promoter region may allow some transcription to occur at a level that is less than if the promoter was completely demethylated. It is easy to see how such a mechanism could add to the overall risk profile of a complex disorder – not only the sequence variation may fine-tune the genetic risk but also the epigenetic variation may be involved.

Additionally, epigenetic variation appears to be modifiable by the environment, and epigenetic changes induced by the environment may be heritable. Changes to the methylation patterns of genes in response to environment have been strikingly demonstrated in rodents, where lack of methylation at the agouti locus promotes yellow coat colour. When pregnant mice are fed dietary supplements containing nutrients such as folate, the distribution of coat colour in the offspring shifts from yellow to brown (Fig. 21.2). Folate acts as a "methyl-donor," and promotes the methylation of the agouti locus with a resultant shift in the coat colour phenotype [17]. Thus, the environment provided by maternal diet has the capacity to alter the phenotype of offspring in the absence of gene sequence change.

Fig. 21.2 *Genetically identical* mice with variable epigenetic states at the agouti locus. *Right*: mouse with unmethylated agouti alleles expressing yellow coat colour. *Centre*: mouse with methylated, and silent, agouti alleles showing the shift to a brown coat colour. *Left*: mouse with a mottled intermediate coat colour phenotype indicating stochastic methylation of agouti alleles during development. Photo courtesy of Professor Emma Whitelaw, Queensland Institute of Medical Research

21.4.5 Epigenetics and Androgenetic Alopecia

Returning to our example of androgenetic alopecia, do epigenetic modifications have any possible impact on hair loss? Epigenetic variation is most striking when looking at phenotypic variation between individuals who have identical genetic make-up. In humans, monozygotic twins are an excellent example, and there is evidence that the development of certain diseases in one identical twin, and not the other, may be due to differences in epigenetic marks [13]. Another way to think about this phenomenon is to consider different tissues in the body of an individual. Each tissue will have the same DNA sequence contained in its cells, but the tissues will have varying gene expression patterns, depending upon what the function of the tissue is. This variation looks likely to be due, at least in part,

to epigenetic differences between the tissue types [33]. Even within tissue types, differences might be seen. On the scalp, there is an established difference in the expression of *AR* between balding (e.g., vertex) and non-balding (e.g., occipital) regions of the scalp in the same individual. *AR* expression is higher in balding scalp hair follicles, leading to the likely assumption that increased *AR* expression levels are important in the development of androgenetic alopecia. This also leads to the hypothesis that the lower *AR* expression levels are protecting the occipital region from hair loss. These two closely lying tissue regions have the same genetic make-up, and even have the same function. So what causes the *AR* expression, and thus hair growth, differences at these two sites? One possible answer is that *AR* is more highly methylated in hair follicles in the occipital region, down-regulating the expression, and research is currently being carried out to test this hypothesis.

Taking this one step further, you will recall that the androgenetic alopecia-associated *AR* SNP was found in a high percentage of men who had no hair loss post-age 50 years. Thus, men who have the same AR genotype have different hair loss phenotypes. It is possible, if methylation is controlling *AR* expression in scalp hair follicles, that there may be methylation differences between affected and unaffected men with the same *AR* genotype. If this is true, then determination of "epigenotype" (the pattern of methylation at the *AR* promoter CpG island) might also be important in determining the risk of hair loss attributable to the *AR* locus.

21.4.6 Complex Genome, Complex Disorders

It is hoped that whilst only a small taste of the complexities of the human genome have been discussed, this section has convinced the reader that dissecting the risk factors of a complex disorder such as androgenetic alopecia is at present a difficult task. In the next section, the concept of "personalised medicine" is discussed. It is presented in the context of what we know about androgenetic alopecia, what there is still left to learn and how this affects current and future capacity for realising the potential to use such knowledge to predict, diagnose and treat common male hair loss.

21.5 Bench to Headside: Translating Genetic Research into Patient Care

21.5.1 Applications of the Genetic Risk Profile of a Heritable Disorder to Patient Care

A common goal of human geneticists is to identify information that can be eventually used in patient care. An understanding of the underlying genetic architecture of a heritable disorder may provide the opportunity for many potential applications. These include:

- Prediction of risk – genotyping risk alleles may allow prediction of the likelihood of developing the disorder in the future
- Prevention – those at high risk according to genotype may have opportunities to avoid developing the disorder all together
- Diagnosis – genotype may provide/confirm diagnosis when differentiation of disorders of similar phenotype, or within-disorder subtypes, is difficult
- Targeted treatment development – knowledge of the underlying genetic abnormalities may provide opportunities to develop new treatment strategies that target these abnormalities
- Pharmacogenomics – often called "personalised medicine," in which the background genetic profile is used to determine the likely efficacy of a drug, or indeed the likely toxicity.

All these possible applications hold promise for translating basic research into clinical outcomes. We shall explore these in the context of their potential in a future "utopic" environment – where the entire risk profile for androgenetic alopecia has been identified. We shall then return to our current state of knowledge and explore these aspects again to illustrate just how far we are from achieving these goals.

21.5.2 The Future: What An Androgenetic Alopecia Risk Profile Might Look Like

We know that the risk of developing androgenetic alopecia is almost entirely, if not entirely, due to heritable

factors. For the most part, these heritable factors will be sequence variation in genes involved in hair growth; however, this may not be the entire story – other types of heritable variation such as gene methylation may also play a role. On the basis of current understanding of the underlying genetic architecture, and of the complexity of the genome, it could be predicted that the risk profile for androgenetic alopecia might consist of genetic variation in a number of genes located in different pathways involved in hair growth and hair cycling. For instance, given that androgens and AR appear to play an important role in risk, it might be discovered that other genes involved in the sex steroid pathway (such as those encoding androgen metabolising enzymes) also harbour genetic variation that contributes to individual risk. Additionally, sequence variation in genes involved in hair follicle cycling pathways might similarly contribute. On top of this, the levels of methylation of promoter regions of some of these genes in hair follicles might add complexity to the risk profile. For the purposes of illustration, let us assume that ten genes are identified, which hold functional variation that contributes to the risk of developing androgenetic alopecia, and for two of these genes, the level of methylation in hair follicles also plays a role in determining risk. Not all genes are necessary for the development of androgenetic alopecia, and from individual to individual, genes involved in one, or both, of the pathways might be affected. However, the number, and combination of these genes that are inherited affect age at onset and severity of hair loss. Such a level of understanding of the causes of androgenetic alopecia would be astounding, and likely not achievable in the near future. However, if geneticists could achieve this, how could this knowledge be used in patient care? We look at each of the five possible applications listed earlier.

21.5.2.1 Prediction of Risk

A simple genetic test that analyses each known risk factor could reveal an individual's risk of developing androgenetic alopecia. For example, if androgenetic alopecia runs in the family, a young man may wish to determine his odds of developing the disorder. Achievement of the level of understanding postulated earlier would provide the opportunity not only to calculate risk based on the inheritance of genetic and epigenetic variants, but would also allow a prediction of the age at which hair loss might be expected to begin, and to what extent it might progress within the young man's lifetime.

21.5.2.2 Prevention

Following prediction of risk, a young man predisposed to androgenetic alopecia might be given the opportunity to prevent his hair loss. Our current understanding of androgenetic alopecia suggests that balding hair follicles eventually shut down completely, and that this state is irreversible. Additionally, current pharmacological treatments for androgenetic alopecia are the most efficacious in men in whom hair loss is less severe [27]. Therefore, prediction of risk may lead to prevention via treatment strategies that are employed earlier, for example, prior to any cosmetically noticeable level of loss or even at the cessation of puberty when the hair loss process is thought to be initiated.

21.5.2.3 Diagnosis

An approach to diagnosing cases of androgenetic alopecia in which the phenotype is ambiguous might be to examine scalp biopsies. However, if the underlying genetic risk profile is known, this could be used to unambiguously determine the pathogenesis of the hair loss. Unambiguous diagnosis is important in the ability of the clinician to provide timely, targeted patient care.

21.5.2.4 Targeted Treatment Development

The exact mechanisms through which the two currently approved pharmacological treatments for androgenetic alopecia, finasteride and minoxidil, exert their effects on hair loss are unknown. For both these treatments, efficacy is variable, and the results are reversed when treatment is stopped [7]. This likely reflects the fact that these treatments are not well targeted to the underlying molecular pathogenesis, and that genetic heterogeneity (i.e. different genetic risk profiles) exists between patients. A thorough understanding of the underlying genetic mutations, including the gene(s)

upon which they act, and their exact action(s) opens up an unprecedented opportunity to design treatments to counteract these mutations. For example, if it were found that the functional mutation in *AR* acts to create an extra transcription factor binding site that increases expression of *AR* in men carrying the mutation, an antagonist of the transcription factor that recognises the binding site might be used to block binding, counteracting the effect of the mutation.

21.5.2.5 Pharmacogenomics

The ability to apply pharmacogenomic or the so-called "personalised medicine" strategies is one of the most anticipated developments that might stem from gene discovery. Pharmacogenomics refers to the field of pharmacology that aims to be able to devise individualised treatment strategies based on underlying individual genetic architecture (Fig. 21.3). The genotype of a patient would be used to determine the most efficacious drug for them, and would also be used to avoid prescribing a drug that might cause serious harm [29].

For androgenetic alopecia, the future of pharmacogenomic strategies might include determining whether or not the hair loss in an individual is caused by variation in genes involved in either the sex steroid pathway, the hair cycling pathway or both. Prescribing treatments targeted at resolving perturbations in the sex steroid pathway to individuals in whom the hair loss is caused by mutations in hair cycling genes is unlikely to be effective. In fact, given the systemic importance of sex steroids, unnecessary disturbances of this pathway may in fact cause harm. On the flipside, prescribing treatments for resolving irregularities of hair follicle cycling genes is unlikely to be efficacious in individuals for whom their genetic predisposition occurs solely within the sex steroid pathway genes. And, prescribing either form of therapy to an individual whose hair loss is caused by perturbations of both pathways is also unlikely to be fully effective.

Taking this one step further, consideration of epigenetic variation may also be important in personalised medicine strategies. If methylation of the promoter region of *AR* does indeed counteract the effect of inheriting the androgenetic alopecia-associated *AR* variant by "turning down" the level of *AR* expression, therapies targeted at resolving *AR* variant effects are unlikely to be effectual in individuals with a highly methylated *AR*. This might also relate to prediction and prevention. There would be no need for a young man who has inherited the androgenetic alopecia-associated *AR* variant, but no other risk factors, to consider preventive strategies such as early adoption of treatment, if he has also inherited a highly methylated hair follicle *AR* promoter.

21.5.3 The Present: What Can We Do with the Current Knowledge?

At present, research has uncovered only a small fraction of the risk profile for androgenetic alopecia. Though we have identified a marker allele of *AR* that occurs in a higher percentage of balding males than their non-balding counterparts, we have not yet identified the exact functional sequence alterations or the consequences of those alterations on *AR* expression. Additionally, we do not know how accurately the marker allele acts as a proxy for the as-yet-unidentified functional variant. Thus, current knowledge is able to provide a somewhat inaccurate indication of only a slight increase in risk of developing androgenetic alopecia. This provides very little predictive or diagnostic value. Until the association of androgenetic alopecia with *AR* is more accurately defined, and other causative genetic factors are identified, gene polymorphism diagnostics will not be possible. This is equally true for other ageing hair phenotypes such as hair graying.

21.6 Conclusions

Hair phenotypes such as androgenetic alopecia and hair graying are controlled, at least to a certain extent, by genes. Therefore, identifying genes relevant to hair phenotypes has the potential to increase our understanding of the mechanisms through which the phenotypes develop, and may be used to predict, diagnose and treat such conditions. However, the genetic risk profiles of such phenotypes are very complex, and may be influenced by the environment. This complexity makes the task of defining genetic risk profiles a difficult one, and one that is yet to be accomplished for any complex human disease or trait. The example we have used throughout this chapter, androgenetic alopecia, is

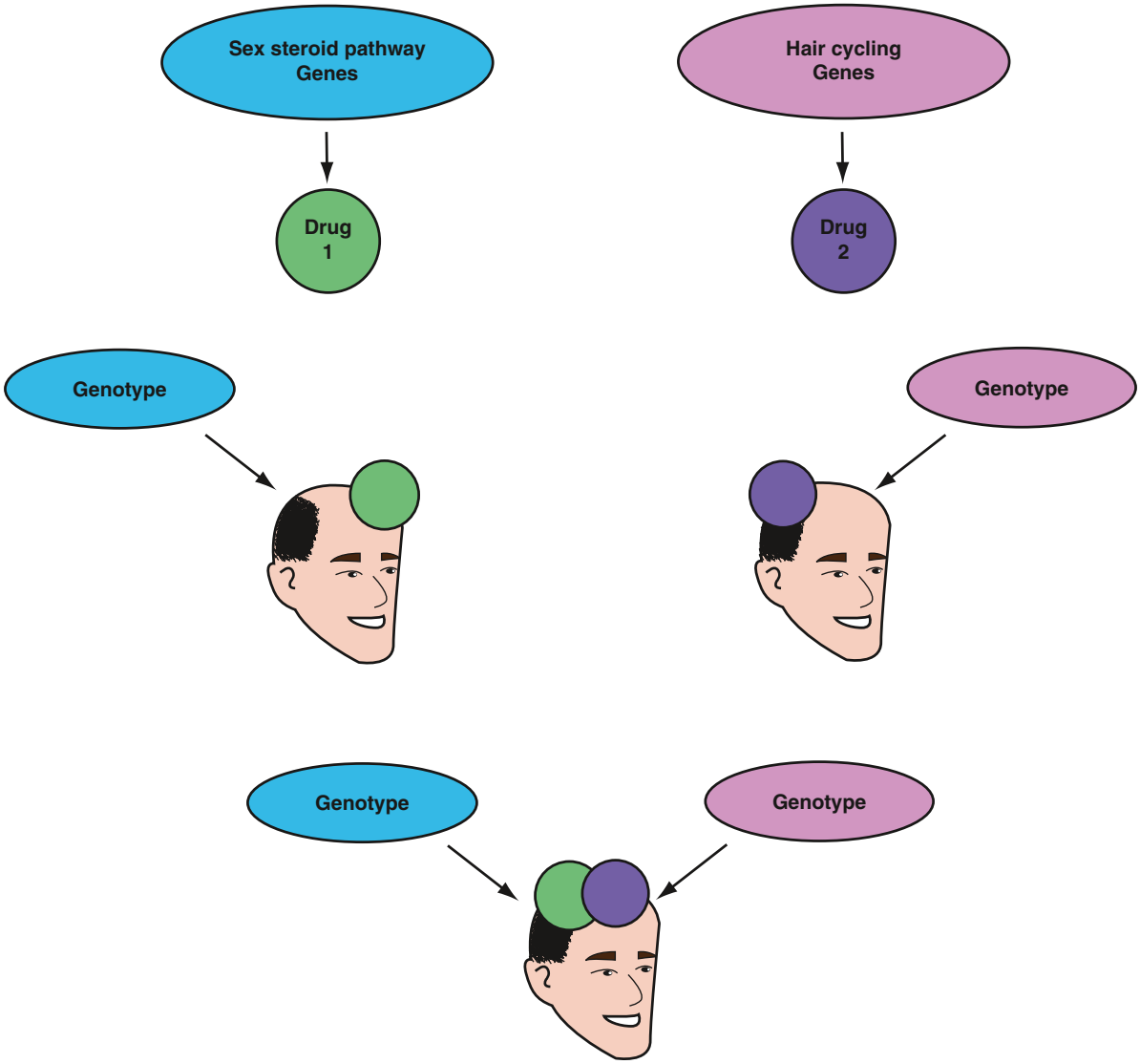

Fig. 21.3 Diagrammatic representation of the future of personalised medicine. On the basis of a full understanding of the genetic architecture of androgenetic alopecia, Drugs 1 and 2 may be developed to target genetic defects in sex steroid genes and hair cycling genes, respectively. By genotyping affected individuals at each known disorder locus, a decision as to whether to treat individuals with Drug 1, Drug 2 or a combination of both drugs can be made

indeed complex in aetiology, and thus far only one risk gene has been identified. At this time, the information we have gained by identifying this single gene is insufficient to allow exploitation for the purposes of diagnostics.

However, there remains much hope on the horizon. The Genome-Wide Association Study (GWAS) approach, has in recent years successfully identified a relative plethora of new loci for numerous human complex diseases and traits. To date, no GWAS has been conducted for hair ageing phenotypes, but it is probably only a matter of time before this occurs. The outcome is likely to be a better understanding of the genetic risk profile of disorders such as androgenetic alopecia, at which time robust gene polymorphism diagnostics based on genetic profile may be closer to reality.

Take Home Pearls

- The genetic aetiology of hair ageing disorders such as androgenetic alopecia and hair graying is extremely complex.
- Currently, our knowledge of this genetic aetiology is very limited, and we are not in a position to accurately use current knowledge to predict, prevent or treat hair ageing conditions.
- However, methods to unravel the complexity of these conditions are evolving quickly, and new approaches, such as genome-wide association studies, may allow us to more fully understand the genetic architecture in the not-too-distant future.
- When this occurs, understanding the genetic aetiology of hair ageing will provide enormous opportunity to develop personalised medicine strategies, whereby preventive measures or efficacious treatments are tailored to the individual.

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Future Directions: The Known and Unknown Roles of Hair-Follicle Stem Cell Types

22

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Core Messages

› Our laboratory has discovered that the hair follicle has both pluripotent and monopotent stem cells. We observed that nestin, a protein marker for neural stem cells, is also expressed in a population of hair-follicle stem cells and their immediate, differentiated progeny. The green fluorescent protein (GFP), whose expression is driven by the nestin regulatory element in transgenic mice (ND-GFP mice), served to mark hair-follicle stem cells and could be used to trace their fate. The ND-GFP hair-follicle stem cells are positive for the stem cell marker CD34 but negative for keratinocyte marker keratin 15, suggesting their relatively undifferentiated state. We have shown that the nestin-expressing hair-follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. In vivo studies show that the nestin-expressing hair-follicle stem cells can differentiate into blood vessels and neural tissue after transplantation to the subcutis of nude mice. Nestin-expressing hair-follicle stem cells implanted into the gap region of severed sciatic or tibial nerves greatly enhance the rate of nerve regeneration and the restoration of nerve function. When transplanted to the severed peripheral nerves or spinal cord of the mice, the nestin-expressing follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. The transplanted mice regain the ability to walk normally. The nestin-expressing hair-follicle stem cells are pluripotent and provide an effective, accessible, autologous source of stem cells for the treatment of peripheral nerve injury and appear to be a paradigm for adult stem cells. We have observed that both the mouse and human hair follicles also contain nestin-negative, keratin-15-positive stem cells that form only keratinocytes as well as the pluripotent nestin-positive keratin-15-negative stem cells. A major question in stem cell and hair-follicle biology is: "What are the main roles of these two types of stem cells in the hair follicle?" Our future understanding of the role of the stem cell types in the hair follicle is a promising approach to study hair growth and aging.

22.1 Introduction

22.1.1 The Hair-Follicle Cycle of Growth, Differentiation, and Death

The hair follicle produces a terminally differentiated keratinized end product – the hair shaft – that is eventually shed. The follicle undergoes cyclical regeneration with at least ten different epithelial and mesenchymal cell lineages [9]. Hair is formed by rapidly proliferating matrix keratinocytes in the bulb located at the base of the growing (anagen) follicle. The duration of

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anagen varies greatly between hairs of differing lengths. Nevertheless, matrix cells eventually stop proliferating, and hair growth ceases at catagen when the lower follicle regresses (telogen). After telogen, the lower hair-producing portion of the follicle regenerates, starting the new anagen phase [9].

Hair-follicle stem cells, located in or near the hair-follicle bulge, possess stem cell characteristics, including multipotency, high proliferative potential, and ability to enter quiescence. Earlier studies have demonstrated that all epithelial layers within the adult follicle and hair originated from bulge cells [9, 10]. Hair-follicle stem cells therefore appear to be responsible for regenerating the hair follicle in each hair cycle.

After wounding, hair follicles form de novo in adult mice. The nascent follicles arise from epithelial cells outside of the hair-follicle stem cell niche, suggesting that epidermal cells in the wound assume a hair-follicle stem cell phenotype. The newly generated hair follicles establish a stem cell population, express known molecular markers of follicle differentiation, produce a hair shaft, and progress through all stages of the hair-follicle cycle [16].

22.1.2 Imaging Hair-Follicle Stem Cells In Vivo with GFP

The insufficiency of markers to identify and track hair-follicle stem cells in the bulge area has hindered the study of hair-follicle stem cells. CD34 expression as first defined by Trempus et al. [33] is a marker for hair-follicle stem cells. Antibodies recognizing CD34 were used to collect viable bulge cells by fluorescent-activated cell sorting [8, 33]. Keratin-15 (K15) is expressed at high levels in the bulge, but lower levels of expression can be present in the basal layers of the lower follicle outer-root sheath (ORS) and the epidermis [23, 35]. A K15 promoter used for the generation of transgenic mice was active only to the bulge in the adult mouse [22].

A breakthrough occurred with the use of transgenic mice, in which the neural stem cell marker, nestin, drove the expression of green fluorescent protein (GFP) (ND-GFP mice). We observed in these mice that nestin was also a marker for hair-follicle stem cells, which suggested that hair-follicle stem cells could form neurons and were pluripotent [21]. The hair-follicle stem cells could then be tracked by their green fluorescence. These relatively small, oval-shaped, ND-GFP-expressing cells in the bulge area surround the hair shaft and are interconnected by short dendrites. In mid- and late anagen, the ND-GFP-expressing cells are located in the upper ORS as well as in the bulge area but not in the hair matrix bulb. These observations show that the ND-GFP-expressing cells form the ORS. Following our report that ND-GFP can serve as a marker for hair-follicle stem cells to track them in the live animal, Morris et al. [26] subsequently used GFP to follow keratin-15-expressing hair-follicle stem cells in transgenic mice. Fuchs' group also subsequently used GFP to identify hair-follicle stem cells and possibly other skin stem cells in transgenic mice [28, 34]. Yu et al. [37] showed that nestin was present in human hair-follicle stem cells confirming our original observation in mice [21].

Li et al. [20] have reported that nuclei from hair-follicle stem cells can be successfully used as nuclear transfer (NT) donors, resulting in live cloned mice. Thus, the nuclei of hair-follicle stem cells can be reprogrammed to the pluripotent state by exposure to the cytoplasm of unfertilized oocytes. These results confirm our results demonstrating the pluripotency of hair-follicle stem cells [21].

The evidence that nestin-expressing cells in the hair-follicle bulge are hair-follicle stem cells rather than a population of stem cells that reside in the hair follicle, whose purpose is to regenerate the neuronal and endothelial components associated with the pilosebaceous unit, is that the nestin-expressing (and GFP-expressing) cells have been imaged over time to regenerate a large portion of the hair follicle as described above [21].

22.1.3 Hair-Follicle Stem Cells Can Form New Follicles and Epidermal Cells

Hair-follicle stem cells from adult mice, when combined with neonatal dermal cells, formed hair follicles after injection into immunodeficient mice [8, 26]. Cultured individually cloned bulge cells from adult mice also were shown to form hair follicles in skin reconstitution assays [8].

As mentioned earlier, Morris et al. [26] used the K15 promoter to drive GFP expression in the hair-follicle bulge cells in transgenic mice. With this model, they showed that bulge cells in adult mice generate all epithelial cell types within the intact follicle and hair during normal hair-follicle cycling.

Taylor et al. [31] reported that hair-follicle bulge stem cells are potentially bipotent, because they can give rise to not only cells of the hair follicle but also to epidermal cells. However, hair-follicle stem cells may form epidermal stem cells only when the epidermis is wounded [15]. Other experiments [27] also have provided new evidence that the upper ORS of vibrissal (whisker) follicles of adult mice contains multipotent stem cells, which can differentiate into hair-follicle matrix cells, sebaceous gland basal cells, and epidermis. Enzymatically degraded skin is also a source of multipotent stem cells, but their relationship with the hair-follicle stem cells is unclear [32].

22.1.4 Blood Vessels Derived from Hair-Follicle Stem Cells

We observed that in ND-GFP mice, skin blood vessels express ND-GFP and appear to originate from hair follicles and form a follicle-linking network. This was seen most clearly by transplanting ND-GFP-labeled vibrissa (whisker) hair follicles to unlabeled nude mice. New vessels grew from the transplanted follicle, and the number of vessels increased when the local recipient skin was wounded. The ND-GFP-expressing blood vessels display the characteristic endothelial-cell-specific markers CD31 and von Willebrand factor [1].

22.1.5 Differentiation of Hair-follicle Stem Cells to Neural and Other Cell Types

ND-GFP hair-follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. These pluripotent ND-GFP stem cells are positive for the stem cell marker CD34, and negative for keratin 15, suggesting their relatively undifferentiated state, as mentioned earlier. The apparent primitive state of the ND-GFP stem cells is compatible with their pluripotency. The ND-GFP hair-follicle stem cells may be more primitive than those hair-follicle stem cells previously isolated [10]. Furthermore, we showed that the ND-GFP hair-follicle stem cells differentiated into neurons after transplantation to the subcutis of nude mice [3]. The pluripotency of mouse ND-GFP-expressing hair follicles has been independently confirmed [25].

Yu et al. [31] isolated a population of stem cells from human hair follicles that express nestin, proliferate as spherical aggregates, and can differentiate into multiple lineages. However, the location of these stem cells was not clear in this study.

22.1.6 Hair-Follicle Stem Cells Can Effect Nerve Repair

When the nestin-expressing hair-follicle stem cells were implanted into the gap region of the severed sciatic nerve of the mouse, they greatly enhanced the rate of nerve regeneration and the restoration of nerve function including the ability to walk. After transplantation to severed nerves, the nestin-expressing hair-follicle stem cells differentiated largely into Schwann cells, which are known to support neuron regrowth. Function of the rejoined sciatic nerve was measured by contraction of the gastrocnemius muscle upon electrical stimulation. The transplanted mice recovered the ability to walk normally [2].

22.1.7 Hair-Follicle Stem Cells Can Effect Spinal Cord Repair

Sieber-Blum et al. [29] showed that neural crest cells grew out when the hair follicle was explanted, resulting in the differentiation to a variety of cell types including neurons, smooth muscle cells, rare Schwann cells, and melanocytes. The location of these cells within the follicle was not determined. Sieber-Blum et al. [30] characterized the behavior of implanted neural crest stem cells from the hair follicle in the contusion-lesioned murine spinal cord. The grafted neural crest cells survived, integrated, and intermingled with host neurites in the lesioned spinal cord. They did not proliferate and did not effect the spinal-cord repair. Subsets expressed neuron-specific beta-III tubulin, the GABAergic marker glutamate decarboxylase 67 (GAD67), the oligodendrocyte marker, RIP, or myelin basic protein (MBP). However, glial fibrillary acidic protein (GFAP) was not detected by immunofluorescence.

In contrast to Sieber-Blum's results, when nestin-expressing hair-follicle stem cells were implanted between the severed thoracic regions of spinal cord in C57BL/6 immunocompetent mice, the spinal cord regenerated and regained function [4]. Two months

after transplantation, GFP-expressing cells had migrated into the joined region of the previously severed thoracic region of the spinal cord. Most of the GFP-expressing hair-follicle stem cells transplanted to the severed spinal cord differentiated to GFAP- and CNPase-positive Schwann cells, a result different than Sieber-Blum [30]. The GFP-expressing Schwann cells formed myelin sheaths, which surrounded the host βIII-tubulin-positive axons, probably enabling them to regenerate. Between 6 and 12 weeks after the hair-follicle stem-cell transplantation, the mice recovered significant hind-limb function [4].

This apparent difference between Sieber-Blum's results [30] and ours [4] is probably due to different cell types transplanted by Sieber-Blum et al. [30] when compared with the hair-follicle stem cells that were transplanted to the lesioned spine in our study. Sieber-Blum et al. explanted the bulge area of a whisker (vibrissa) in vitro. Within 3–4 days, cells migrated from the explanted bulge area and grew on the surface of the culture dish. Glial markers were not expressed or were expressed only at low levels in the migrating cells. Four days after onset of migration, these cells were harvested and further expanded in culture for another 4 days. After 4 days of expansion, the cells were implanted in the lesioned spinal cord. Although neurons and oligodendrocytes formed after transplantation, glial cells did not appear, which are apparently critical for spinal cord regeneration.

Our approach was to actually isolate vibrissa stem cells, culture them for 2 months, and then implant the cells in the lesioned spinal cord. In contrast to Sieber-Blum et al. [30], in our study [4], the vast majority of the implanted cells (82%) formed glial cells in the lesioned spinal cord. Our hypothesis is that the glial cells promoted axon growth and recovery of spinal cord function. Perhaps the outgrowth method of Sieber-Blum et al. [30] did not allow for recovery of sufficient numbers of cells capable of glial differentiation, which in turn did not allow for sufficient axon growth for spinal cord recovery.

Miller's laboratory observed that cells they called skin precursors (SKPs), found among cells released from enzymatic digestion of dermis, could form myelinating Schwann cells when injected into the injured sciatic nerve [24], which is similar to our earlier results with nestin-expressing hair-follicle stem cells [2]. The same laboratory then showed that SKPs could promote spinal cord repair. The SKPs were released from skin by collagenase treatment of the dermis, which produced a mixture of cells [7]. The origin of SKPs within the skin is thus unclear. In contrast, our results show that nestin-expressing hair-follicle stem cells, a defined population, can functionally repair the severed spinal cord. It should also be noted that our studies as well as the studies with SKPs used fluorescent proteins to track the transplanted cells, a technology pioneered in our laboratory [6, 12, 14, 18, 36].

22.1.8 Location of Pluripotent and Monopotent Hair-Follicle Stem Cells in the Mouse

We have recently shown in the mouse that ND-GFP expressing stem cells are located in the upper hair follicle immediately below the sebaceous glands just above the hair-follicle bulge area. The ND-GFP stem cells are K15-negative. The nestin-positive, K15-negative stem cells in the mouse hair follicle are pluripotent and can differentiate to neurons, glial cells, keratinocytes, and other cell types, as mentioned earlier. Nestin-negative, K15-positive cells, on the other hand, are located in the bulge area of the mouse hair follicle and can differentiate only to keratinocytes [5].

22.1.9 Location of Pluripotent and Monopotent Hair-Follicle Stem Cells in the Human

In the intact human hair follicle dissected from the scalp, the cells immediately below the sebaceous glands just above the bulge area were also observed to be nestin-positive, K15-negative. In contrast, the hair-follicle bulge area contained nestin-negative, K15-positive cells, similar to the mouse [5].

The intact human scalp hair follicle was divided in three parts (upper, middle, and lower). Hair follicle cells that were located immediately below the sebaceous glands just above the bulge area in the upper section of the intact scalp hair follicle were isolated. The isolated stem cells were suspended in DMEM-F12 containing B-27 supplemented with bFGF every 2 days. These nestin-positive keratin-negative cells formed spherical colonies in this medium, which were termed hair spheres by Yu et al. [37]. Ten days after switching the medium to RPMI 1640 containing 10% FBS, differentiating cells were observed migrating away from the colony. These differentiated cells included β3-tubulin-positive neurons, S-100-positive

and GFAP-positive glial cells, K15-positive keratinocytes, and SMA-positive smooth muscle cells [5].

In contrast, the plucked scalp hair follicle, which did not contain sebaceous glands or nestin-positive, K15-negative cells above the bulge, was divided to three parts (upper, middle, and lower). The upper part of the plucked scalp hair follicle, which contained the bulge area and nestin-negative, keratin-positive cells was suspended in DMEM-F12 containing B-27 supplemented with bFGF every 2 days. Ten days later, the upper part of the plucked hair formed hair spheres. The proliferating cells were identified as keratin 15-positive keratinocytes. After switching the medium to RPMI 1640 containing 10% FBS, keratinocytes were formed but neurons and other nonfollicular differentiating cell types were not observed. The middle and lower parts of the plucked hair follicle did not proliferate in DMEM-F12 containing B-27 and bFGF [5].

22.2 Conclusions

Thus, the hair follicles of mice and men appear to have two populations of stem cells: a pluripotent type and an apparent unipotent type. These stem cells have important potential for regenerative medicine and hair growth, respectively. Thus, the hair follicle may be the most useful of adult stem cells due to easy access and differentiation potential.

We have shown that the hair-follicle bulge area is an abundant easily accessible source of actively growing pluripotent adult stem cells that could serve a clinical source in humans. The availability of the ND-GFP mice has enabled the identification, isolation, and characterization of these highly pluripotent hair-follicle stem cells. These hair-follicle stem cells express the stem cell marker CD34 and nestin but are negative for the keratinocyte marker keratin 15, indicating their relatively undifferentiated state. The hair-follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. In vivo studies show the nestin-driven GFP hair-follicle stem cells can differentiate into blood vessels and neural tissue after transplantation to the subcutis of nude mice. Nestin-expressing hair-follicle stem cells implanted into the gap region of severed peripheral nerves or the severed spinal cord greatly enhance the rate of nerve and spinal cord regeneration and restoration of function. After transplantation to the severed nerve or spinal cord, the follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. The transplanted mice regain the ability to walk normally. Thus, nestin-expressing hair-follicle stem cells provide an effective, accessible, autologous source of stem cells for treatment of peripheral nerve and spinal cord injury.

The nestin-expressing hair-follicle stem cells thus have the potential as an alternative to the use of embryonal stem cells or fetal cells for regenerative medicine. The hair-follicle stem cells do not have the ethical problems that embryonal or fetal stem cells have. Even more important, the hair-follicle stem cells are much more easily accessible than these other stem-cell types and offer the potential for autologous treatment, as they can be readily expended in culture after isolation from the patient. The fact that we [5] and Yu et al. [37] have shown nestin expression and pluripotency of human hair-follicle stem cells further suggests the clinical potential of hair-follicle stem cells for regenerative medicine.

It is also important to note that the dermal papilla is a potential source of multipotent stem cells that may have use in regenerative medicine. For example, Jahoda's group has demonstrated that hair-follicle dermal cells repopulate the mouse hematopoietic system [19], can differentiate into adipogenic and osteogenic lineages [17], and participate in wound healing and induction [11].

Hair-follicle stem cells also have great potential for hair restoration [8, 13, 26]. Our future understanding of the role of the stem cell types in the hair follicle is a promising approach to study hair growth and aging.

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Future Directions: Bioengineering the Hair Follicle

23

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Core Messages

› Considered here is the rationale for cellular engineering of the hair follicle: the cells needed, the mechanisms of hair regeneration from dissociated cells, current successes, immediate challenges, and future vision. As evidenced by its growth cycle, the hair follicle has the ability to regenerate. This property presents an opportunity to the cellular engineer for reconstructing hair follicles where needed. The field has demonstrated great success with laboratory mammals. Translating that success to human adult cells, and thus the clinic, is the current challenge to our mechanistic understanding and application.

23.1 Introduction

Even before the poet mentions the cavernous cutaneous wrinkle of age, he describes the "balding pate" or the "hoary hair." Hair, the great communicator, is perceived to signal age and is thus a cosmetic focus for the maturing individual, particularly in our youth culture. Since the pigmentary changes occurring with age and the role of hair transplantation in hair restoration have been covered by other authors in this volume (see the discussions by Drs. Tobin and Peters, and Drs. Shapiro and Finner, respectively), we focus here on the replacement of hair using a futuristic approach: the generation of new hair follicles from hair follicle-derived cells. We will consider here the rationale for cell-based therapy, the cells needed, the mechanisms of hair regeneration from dissociated cells, current successes, immediate challenges, and future vision.

For those of us in the hair regeneration field, it is our collective goal to replace/regenerate new cycling permanent hair follicles in a region of skin, which the patient perceives is wanting of hair. As mentioned earlier in this text, hair transplantation is one solution; it has become a fine-tuned, highly sophisticated discipline far more efficient from what it was when first suggested [22, 63]. The problem with hair follicle transplantation, though, is the limited donor supply.

Although the ultimate goal for hair loss is prevention, an ideal therapeutic approach we seek for the balding patient would consist of (1) a single topical chemical application, (2) a defined physical stimulus, or (3) a cellular implantation. All three approaches would appear to require the reengineering of resident hair follicle cells in the skin. Recent work suggests that such a vision could be realizable for any one of the three approaches [28, 31, 81]. On the chemical side, recently, Yamanaka [51, 82] showed that skin fibroblasts could be transformed into pluripotent cells (iPS cells) by ectopic expression of four important stem-cell genes. Although not yet applied to the skin, this work suggests that cell lineages can be induced by specific laboratory genetic manipulations. So, given the correct combination of gene expression, we might be able to induce inactive skin cells into active hair follicle-inducing cells, such as by activating the expression of the appropriate transcription factors [97]. Subsequent to the Yamanka report, Huangfu et al. [28] suggested that, if

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one knows the important genes and thus the relevant molecular pathways, one might be able to induce the same effect without or with minimal gene manipulation by using highly specific small chemical moieties. Although modest in their effect, we recognize with relief that a few pharmaceutical entities do indeed stimulate hair growth (minoxidil, finasteride, and latanoprost). On the physical side, it has been shown recently, in a carefully documented report, that a full-thickness skin wound in the mouse will repair with the regeneration of new hair follicles [31]; moreover, stimulating the Wnt growth factor pathway in that system enhanced new follicle formation and blocking this pathway reduced new hair follicle formation. On the cellular side, the approach would isolate trichogenic (hair forming) cells from the patient, expand the cells in culture, and inject the cells into skin. The cell engineering effort is being pursued by many laboratories with the goal of reconstructing life-essential organs (e.g., formation of liver, kidney, and pancreatic islets [4]). As we have spent some time trying to understand how the hair follicle can be restructured from dissociated cells alone, we will focus here on this topic. We also recognize that the principles learned from the hair follicle will be applicable to other complex organ systems.

23.2 Hair Follicle Morphogenesis, Hair Cycle, and Stem Cells in the Skin

The hair follicle arises within the primitive epidermis involving complex epithelial and mesenchymal interactions. The first indication of hair follicle formation in fetal skin is a distinctive orientation of the primitive epidermal cells, which is also declared by specific markers (e.g., Ep-CAM [77], CD10 [66], and others [3]). This is followed by condensation of the deep lying dermal cells and a finger-like downgrowth of the epithelium to join and encircle the dermal condensate. Once fully formed, the follicle, a multilayered cylindrical structure, undergoes a growth cycle consisting of phases of increasing organ size with shaft formation, structure regression, organ rest, and, finally, shaft shedding [80]. Folliculoneogenesis as well as hair cycling requires constant and intimate epithelial–mesenchymal contact and interactions to cycle and grow [49, 62]. That the lower portion of the anagen follicle is actually consumed in the regressing process signals the regenerative power inherent in this structure, a property that persists throughout life – from the youngest to the oldest individual. Thus, the hair follicle houses within it the elements of regeneration, features shared by few other organs in the mature mammal. How can we capture this hair follicle trait and use it to produce new hair follicles wherever and whenever we want; moreover, might we be able to use the hair follicle to help regenerate other organ systems (e.g., the tooth, Yu et al. [93], or bone marrow, Lako et al. [41])

To effect? the complete hair cycle, we would assume that there are potent regenerative cells in the region of each hair follicle. In fact, within and surrounding the hair follicle are niches housing epidermal, dermal, hair follicle, sebaceous, melanocytic, neural, and mesenchymal stem cells (MSCs) [14, 18, 20, 79, 86, 91]. In this discussion, we are using the term “stem cell” to describe cells that have the potential for self-renewal and an ability to differentiate into a specific, committed, developmental pathway [72]. To exploit these cells for clinical needs, we must understand them and the regions in which they rest.

In a serendipitous landmark experiment, Cotsarelis et al. [15] showed follicular epithelial stem cells in the hitherto little-appreciated hair follicle bulge. While the bulge is considered to house epithelial cells critical to hair follicle cycling, such cells may also contribute to the regeneration of other surrounding structures in a wound/repair situation [30, 42, 44]. Lineage studies showed that these cells migrate up and down the follicle and can give rise, under the proper circumstances, to most epithelial cells of the skin: the epidermis, sebaceous gland, and hair follicle [65]. Cells of the bulge have been isolated and characterized from mouse [7, 55] and man [37, 61]. By gene expression, these cells show downregulation of genes related to cell division and hair follicle differentiation (e.g., Wnt family of genes) and upregulation of genes related to immunosuppression (e.g., CD200). These cells have the potential to generate whole skin structures, so when they are dissociated, purified, and combined with inductive dermal cells, new pilosebaceous structures form [7, 55]. To successfully engineer a follicle, then, one would need such epithelial and dermal hair follicle progenitor cells.

Dermal stem cells were recognized long before workers in the field appreciated the central role of the bulge (epithelium) in follicular biology. In the period between 1960 and 1980, a small group of workers demonstrated, in now classical studies, that the mesenchymal portion of the hair follicle, the follicular papilla,

or dermal papilla, alone, when properly placed adjacent to an epithelium, could induce new hair follicle structures (in rodents [13, 62, 88]). These experiments involved dissecting papillae from anagen hair follicles and placing them either below a proximally truncated whisker follicle (which would not grow in the absence of papilla) or in the superficial-most skin [62]. The dermal papilla cells responsible for this property could be expanded in culture with retention of hair follicle inductive activity [29, 33, 90]. Recent studies have demonstrated that these dermal cells also have very special plastic regenerative properties beyond that of the hair follicle. Papilla cells have been shown to have MSC properties, and they can be induced to differentiate into adipogenic, osteogenic [34], and hematopoietic cell lineages [41]. Moreover, a subpopulation of connective tissue sheath and papilla cells express *nestin*, an intermediate filament protein, and transcription factors, *slug*, *twist*, *snail*, all markers of neural crest stem cells that are capable of neural and Schwannian differentiation [1, 2, 18]. While it would appear that epithelial cells and dermal cells together are needed to engineer a hair follicle, recent work suggests that one stem cell of the follicle has the properties to undergo dermal to epidermal transformation [16], suggesting the possibility that one precursor cell type might be able to handle the whole job.

23.3 Generating Hair Follicles from Skin Fragments

Attempts have also been made to generate new pilosebaceous structures by inserting fragments of the hair follicle into skin. Previously, we discussed the spectacular effect of implanting whole dermal papillae, but whole follicle fragments have also been used. Implanting bisected portions of hair follicles, however, have met with mixed results. In general, it is found if a hair follicle is sectioned above the bulge horizontally, the lower portion, when properly placed, will regenerate a whole follicle; in contrast, the upper half, above the bulge, rarely [84], if ever grows [70]. From such studies, it appears that, if the hair follicle fragment contains the bulge, a regrowth event may be observed. Overall, these studies are difficult to evaluate because of inadequate quantitation, but it is clear that bisecting follicles does not augment follicle number: at best one new follicle results from one donor follicle fragment.

Hair follicle cells have been used by many workers to generate a skin equivalent [19, 27, 47, 85]. Several studies have found follicle-like aggregates in their constructs but in no case have defined follicles been formed using human cells [26, 47, 48]. In one fascinating report, whole human vellus hair follicles were transplanted into a long-lived skin equivalent. This preparation was demonstrated to be useful in evaluating the effects of the pilosebaceous unit on percutaneous absorption [54].

23.4 Generating a New Hair Follicle from Dissociated Cells, In Vitro

Early studies reported that *fragments* of fetal tissue would continue to develop in vitro if placed in the proper environment. In fact, cultures (hanging drop) of mouse fetal skin will mature to form skin and hair follicles [23, 24]. Recognizing that new organ formation in the embryonic/fetal environment does not normally arise from dissociated cells, at the outset one must question if it is possible for any mature biological structure to form starting with nonadherent, free-floating unorganized cells.

At least 50 years ago, a community of embryologists asked this question and tried to reform organs from trypsin-dissociated cells. Using cells derived from fetal skin, they discovered to their delight and bedazzlement that structures could and did form. Moreover, they learned that, under the proper conditions, such cells have the inherent ability to recognize and adhere to the same cell type (be they from fetal mouse or chick, mixed species or pure) and reform organs [5, 56–58]. So, cells – at least fetal cells – apparently remember to what organ they belong and will reassemble that organ appropriately (given a suitable milieu and time).

Heretofore, it has not been possible to regenerate a complete mature hair follicle in vitro when starting with a suspension of dissociated cells. The situation may be different if such cells are injected into a piece of skin in vitro. In one study, when dissociated trichogenic cells (culture expanded human DP and ORS cells) were implanted into long-term skin organ cultures of human-retroauricular skin and maintained in culture for up to 8 weeks, new hair follicles were believed to have formed. Incorporation of labeled donor cells into hair follicles of the recipient skin was documented in this study [38], although the possibility

that the donor cells might have incorporated into existing follicles was not excluded.

23.5 Generating a New Hair Follicle from Dissociated Cells, In Vivo

In 1992, the NIH group [46] tested if dissociated cells could be used to produce completely mature hair follicles. They combined epidermal and dermal newborn mouse skin cells and placed them within a chamber [89] secured under the skin of immunocrippled mice (*nu/nu* or *SCID*). They found that the cells settled on subcutaneous fascia, reaggregated, and by 3 weeks, produced mature hair follicles with outgrowing hair shafts. The Lichti system has proven to be very useful for many studies testing if isolated or molecularly altered cells are able to reform hair (e.g., [67, 73]). An adaptation of the Lichti system was presented by Qiao et al. [68]. In the Qiao assay, the cells to be measured are placed under the dermis on top of an inverted epidermal equivalent. After 4 weeks, the skin is uplifted to reveal newly formed skin with associated hair follicles and shafts exposed to the surface.

The latter systems, although useful, have some substantial drawbacks: (1) a large number of cells needed to execute the assay, (2) a long time required to obtain a result (3 weeks), (3) a limitation of the assay to one animal at a time, and (4) the difficulty in teaching its set up and execution. To make the assay more facile, Zheng et al. [95] simply injected trichogenic cells into the dermis and observed the formation of mature hair follicles. This system, referred to as the "patch assay," because of the colored appearance of the gross skin overlying the newly formed follicles (the donor cells arise from a pigmented mouse), has proven to be faster (8–10 days), requiring less cells and less animals (eight assays can be performed on the truncal skin of one mouse). Critical to the success of this assay are the trichogenicity of the cells used and the ability of the cells to aggregate in the injection site. It is notable that the follicle formed in this system has all the layers of a mature mouse follicle, has sebaceous glands, produces a mature shaft, and it cycles. The limitation of this assay is that the follicles form deep under the epidermis, and the newly formed shafts do not penetrate the skin surface; this spatial restriction limits hair shaft disposal so that the new follicles are essentially destroyed after the second cycle. Because of its ease of use, however, this assay is an ideal format for quickly measuring the trichogenic activity of a large number of different cellular populations.

The success of the above systems is, in part, because *mouse newborn* skin cells are used in the construct. When *human adult* cells are used alone, follicle-like structures may form. Greater success with human cells has been achieved only when one of the two donor components (epithelial or dermal) is human, but not both (the complementary cells arise from neonatal mouse). Such a result has been reported [17, 96]. In the Ehama study, when neonatal human keratinocytes were cografted with murine dermal papilla cells, hair follicle-like structures formed. In the study of Zheng et al., chimeric follicles formed when cultured human dermal cells were injected (in a patch assay) with neonatal mouse epidermal cells or when cultured human epidermal cells were injected with neonatal mouse dermal cells. Why human cells are so recalcitrant in this regard remains one of the challenges of the field.

Since ultimately we would want to reproducibly generate in the laboratory new hair follicles using human cells, we must establish those criteria which define a hair follicle. In an attempt to meet this need, Chuong et al. [11] suggested a set of criteria to which investigators could aspire. The criteria defined a hair follicle as a structure with proximal distal organization, concentric layers of cells typical of the sheaths seen in vivo, unique shaft product, sebaceous gland, inherent ability to cycle, and demonstrated regeneration of a new proximal follicle with each cycle. By these criteria, the structures generated within these systems [46, 95] were hair follicles, indeed.

23.6 Generating a New Hair Follicle from Dissociated Cells Involving In Vitro and In Vivo Steps

Once the proper trichogenic cells are generated and stabilized, they must be delivered into a receptive site. As mentioned earlier, Zheng et al. [95] found in their early work on developing the patch assay that it was critical to keep the cells densely packed, apparently to support sufficient cell interactions for neogenesis. They found that, if the cells were implanted diffusely, and thus in low density along a facial plane, few defined structures developed. One obvious way of assuring appropriate cell–cell interactions is to form hair follicle progenitor structures in vitro by means of cell aggregation technology and then deliver in vivo. This procedure is looked upon as a way of assuring new

organ formation, but also gives the surgeon greater control in patterning.

Cell aggregation is not a strange occurrence in hair follicle biology in that papilla cells in culture typically form mounds early in the culture [53], and dermal cells are stabilized in vitro if they are kept in, and grow as, aggregates [64]. One way of making hair progenitor cell constructs has been by means of aggregates. Isolating trichogenic cells and then reaggregating them in vitro by a rotation and flotation procedure allowed the formation of organoids, which then completed development in tissue (grafting) [83]. Qiao et al. [69] isolated follicular dermal and epidermal cells from embryonic mouse skin (E18), formed aggregates, grew the aggregates in culture, and then transplanted these constructs into immunocompromised mouse skin showing the formation of new hair follicles. Because the aggregates developed into hair-like structures, they were referred to as "protohairs" [69].

The real challenge has been to form human hair follicles from dissociated human cells. As mentioned earlier, the hybrid or chimeric situation works where a system of half human and half mouse cells are used. While human epidermal and dermal cells alone do not readily generate follicular structures, human epidermal cells plus newborn mouse dermal papilla cells did result in follicle-like formations [17]. The difficulty of generating hair follicles in the patch assay using only human cells (note that in virtually all studies the human cells are derived from adults) has been encountered by other workers [17, 95]. They found, although, using the chimeric or hybrid assay, that when one component is newborn mouse, follicles form which fulfill the proffered criteria. The first attempt to reconstruct a follicle using adult cells was presented by Reynolds and Jahoda [74] using adult rat and adult human anagen follicle cells. The cultured epidermal and dermal cells were packaged into the collagenous shell of a rat vibrissa follicle and then placed in culture. After 2–3 weeks, irregular but recognizable hair fibers formed from rather ill-shaped follicle bulbs.

23.7 Mechanism of New Follicle Formation from Dissociated Cells and Origin of Constitutive Cells

A cellular engineering approach entails the expansion of trichogenic cells in tissue culture and then implanting them into mature skin. The resultant hair follicles are thought to arise by one of two different mechanisms, each illustrated by studies in the mouse [81].

The first involves the formation of completely new structures, folliculoneogenesis. The mechanistic steps were elucidated by Zheng et al. [95] using the patch assay and a step-wise differential description of cell changes. Over a 14-day observation period, it was reported that new follicles formed by day 8. The initial event was epithelial cell aggregation with apoptosis predominately in the center of the cluster leading to "follicular cyst" formations. Dense dermal-cell clusters (alkaline phosphatase positive) were found at specific sites on the periphery of the epithelial condensates, and from these points, follicular buds, pegs, and full follicles arose. With time, a follicle formed with characteristic layers, sebaceous gland, and shaft; moreover, cycling could be demonstrated immediately after the full follicle appeared. Epithelial organization was seen earliest, acting as a platform from which follicles arose. The implications drawn from this study challenge, but do not prove the past and current notion, which states that the first inductive signal for hair follicle formation arises in the dermis [25].

A second mechanism has been suggested by the work of McElwee et al. [52] (discussed by Jahoda [32], and observed by us, (unpublished observations) where, after cell injection, there appears to be a conversion of small hair follicles to large. In this study, cultures of whisker dermal papilla cells were implanted into ear skin. With time, there was a switch from the fine hair of the pinna to coarse hair, more typical of vibrissae; moreover, implanted cells were found to be incorporated within the papilla and connective tissue sheath of the larger hair follicles. The notion that injected dermal cells might have the ability to incorporate and thus transform a small to a large hair follicle suggested the possibility that, in humans, injecting such cells may implement a switch from vellus to terminal hair follicles [81].

In the process of new hair follicle formation - either neogenesis or switch - does the host contribute cells to the reforming structure? Although we envision that the cells we implant will generate the lion's share of the new hair follicles seen, it is not yet clear in any of these systems to what degree the host contributes cells to the neofollicles. It has been shown that progenitor (stem cells) cells arising from the bone marrow circulate and, in some cases, are found to play a role in wound repair/organ regeneration, and even appendage

formation [8, 45, 72]. Such circulating stem cells home to sites by chemoattractants such as stromal-derived factor-1 (SDF-1, binding to the cell surface chemokine receptor, CXCR4), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), or fibroblast growth factor-2 (FGF-2) [12, 39, 71]. In mice, there is direct evidence that bone marrow cells may contribute to wound repair. When tagged bone marrow cells (green fluorescent protein, GFP, transgenic mouse) are mixed with embryonic mouse skin cells and transplanted onto skin defects made in the back of an adult recipient mouse, fully differentiated skin results within 3 weeks, with incorporated GFP positive cells in the epidermis, hair follicles, sebaceous glands, and dermis [36]. So, the host may make a contribution to the follicles formed, but the extent of this contribution needs critical study.

23.8 Challenges for Folliculoneogenesis in the Clinic and the Aging Patient

While laboratory studies of rodent folliculoneogenesis have been dramatic, the most successful examples have been done using cells derived from *newborn or fetal* animals, either as a population of cells arising entirely from a newborn animal or newborn animal cells complementing adult cells (hybrid or chimeric, e.g., [23, 24, 83, 92, 95]). Reformation of hair follicles from adult cells has been observed to be incomplete and, in general, disappointing. What is lost in the adult?

With age, there is a decrease in the number of progenitor cells. In mouse, there is a drop in bone marrow stem cells from 6- to 10-fold between the first and twelfth months of life [40]. In human bone marrow, it is estimated that the population of MSCs decreases from 1 per 10^4 cells in the newborn to 1 per 2×10^6 cells in an 80-year-old individual [9]. In older patients, will there be enough robust regenerative cells to expand and use clinically?

For reasons we do not yet understand, adult cells of any species are strikingly recalcitrant to form new hair follicles from dissociated cells. Since follicle regeneration is most commonly demonstrated in an immunocompromised mouse model, we asked the question if trichogenic cells are prevented from forming a new organ because the recipient host is not immunocompromised enough. Using immunodeficient recipient mice and three donor species (mouse, rat, and dog), we found that, as long as the cells arose from fetal or newborn animals, new cycling hair follicles formed. We concluded that the recipient animal is fully capable of xenograft organogenesis as long as the cells are potent (notably, in the same environment *adult* cells will not form full mature follicles) (Nace et al. unpublished observations). Other studies of skin regeneration have actually come to the same conclusion, namely, that regeneration is a property of young cells and that the property travels with the cells and not with the recipient milieu [50]. This raises the question if regeneration can be effected using adult cell populations at all. Clearly, the naturally cycling hair follicle in the aged adult tells us that cells with regenerative properties persist in the adult but how to get at them is the challenge.

It remains an objective to transfer the incisive work done with the newborn mouse model to the clinic. One dramatic study with unexpected results, and not yet repeated in a second laboratory, describes [75] the success of transplanting human dermal sheath fragments from an adult male donor into the arm of an adult female recipient and generating a large, heavily pigmented hair shaft. Since laser capture microscopy analysis showed that the dermal cells possessed Y chromosome, it was concluded that donor cells from the male were incorporated. The inductive power of these allogeneic human cells presents a paradigmatic breach, as yet, not fully explained.

23.9 Future Challenges and Expectations

Because of the success of the animal studies, experience indicates that generating new hair follicles from human cells in human skin will be accomplished in the near future. While forming such follicles in a robust fashion in an industrial setting will be one major accomplishment worthy of some celebration, anticipated thorny challenges will remain.

The first is controlling hair growth patterning. Pattern formation is an aspect of biology attracting much attention [6]. Some recent work has focused on the hair

follicle. We recognize that the hair follicle like the skin itself shows marked variation from site to site; no two hair follicles are alike (excepting bilateral symmetry) differing in length, curl, texture, surface, color, and androgen sensitivity. This difference has been ascribed to a chemical gradient of stimulating and inhibiting molecules first described as a reaction–diffusion system [60, 87]. Recent studies implicate several molecular lineages in follicle patterning: homeobox genes, ectodysplasin, and Wnt.

Homeobox genes have been considered to be important in hair follicle patterning [10, 35]. Work suggests that stem cells have positional memory, and this property can be ascribed to patterning genes, such as the homeobox gene family group. Expressed by the progenitor cells, these genes give rise to patterns that reflect the site of progenitor cell origin [43]. For hair transplant surgeons, that conclusion would not be a surprise since the efficacy of the Orentreich approach was built on the androgen insensitive cells of the donor site. The stability of the follicle pattern is important in the latter case, but for other applications such as transplanting a scalp hair follicle to an eyebrow region or a scalp hair to the pubic region, the stability of donor pattern would be less attractive. From a detail genomic study of fibroblasts from multiple skin sites, Rinn et al. [76] conclude that the gene expression pattern of fibroblasts are unique to the area from which the cells arise, so it would appear genomic manipulation of some sort will be needed to meet the clinical application.

Evidence has been presented that ectodysplasin-A1 is among the key regulators of pattern formation in the skin [94]. Mou et al. [59] report that ectodysplasin receptor (Edar) and bone morphogenic protein (BMP) acts to produce a pattern by means of an activation-inhibition mechanism. There is a fundamental epithelial to dermal interaction involving Edar feedback from the epidermis coupled with induction of dermal BMP4/BMP7 in the dermis. The BMPs, in turn, act back on the epidermis to block Edar and repress follicle formation. Direct evidence also implicating the Wnt pathway in follicle patterning has been presented [21, 78].

A second challenge, from the manufacturers' perspective, is to be able to use allograft cells in a cell product. This would allow the availability of an "off the shelf" product, which could be adapted to any patient. The first but not insurmountable challenge here is infection. More difficult is the character of the hair shaft, a patterning problem: as we noted earlier, follicles show great variability within a given person, let alone between people. Perhaps, by using autografts close to the region of therapy, the aforementioned problems could be minimized.

A third challenge, the central theme of this book, is the potential aging of a newly formed follicle. Is there an inherent lifetime for a given hair follicle? Why does hair become thinner with age? Will the aging phenomenon also impact the lifetime of neofollicles? Understanding the molecular pathway of "aging" will be needed to fully develop cell-based therapies.

The opportunities for new organ formation are the future of medicine. We look forward to this accomplishment in the near term – in particular for the hair follicle and skin – but recognize that the feat will be achieved in stages, like the metamorphosing insect, who declares his ultimate beauty only after successfully completing each arduous, energy-expensive, time-demanding step.

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Hair After Death

24

Andrew S. Wilson and Desmond J. Tobin

Core Messages

› The hair follicle, for all its highly complex morphogenesis and life-long cycling, generates individual fibers that can (given the right conditions) persist long after the death of their host, about whom they can continue to tell tales. Much of this robustness is embodied by the unique physicochemical structure of the hair shaft which limits any significant post-biogenic change. This chapter outlines the value of hair to both archaeological and forensic investigation, specifically highlighting the significance of the incremental rate of hair growth. This property enables retrieval of detailed time-resolved information for changes in diet and physiological change, toxicology, exposure to pollutants, and use of controlled substances, in addition to individualisation using DNA.

24.1 Introduction

The scientific investigation of "Hair after Death" is one of the very few areas of human science where we can be forgiven for being a little…duplicitous, as hair fibers are "as dead" on the most active and alive scalp as they are after we have long-since passed away. By the time the hair follicle forms the keratinized hair shaft within its follicular factory, the fiber itself is effectively "dead." Biologists even refer to this process as "terminal differentiation" to hammer home the point [114, 115].

This chapter will largely focus on the terminal hair of scalp and on the fact that as the hair shaft emerges from the body it provides both a permanent record and a time-resolvable snapshot of the conditions within the body at the time of keratinization. Given that hair grows at a constant rate, this then offers a unique record of an individual's "lifeways" information [115, 127]. This is currently unavailable from any other body tissues, and as such, is of relevance to both the living and in reconstructing information once individuals die. Several key characteristics of hair growth make this a particularly attractive tissue for scientific study in archeology, forensic science, and with a potential for modern clinical benefit also. There is currently a high level of interest in the hair fiber as a biomonitor [116] of a range of exposures both during life and after death, and it is gratifying to see how what was often viewed by scientists and physicians as little more than detritus has since become something of an archive of the body's life and death experiences. Much of this relies on the unique development of hair via its highly dynamic, cyclic process of growth in which the duration of these growth cycles depends on a myriad of factors including from the more general (e.g., body site) to the most individual (e.g., age, nutritional habits, hormonal factors, and exposures) [104].

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Here, we will take the reader through some general issues relating to postmortem tissue change, inclusive of the hair follicle, followed by discussion of the biogenic change in the hair fiber associated with the nature of hair fiber growth and the survival of recovery of hair from different depositional environments. We will then provide comment on the significance of hair in the forensic sciences, its biogenic information and associated taphonomic implications (i.e., decomposition, postmortem transport, burial, compaction, and other chemical, biologic, or physical processes that affect the hair remains).

24.2 General Postmortem Change to Tissues

Our intimate personal relationship with our own mortality encourages us to view death as a single event identifiable at the critical time point. However, we also joke at being "born with the terminal disease called life!" The latter is actually much more accurate than the former view. However, when the life history of the hair follicle is considered in depth, the passing of the life force at the organismal level (viewed conventionally as irreversible heart–lung–brain failure) can be taken at the relevant point to begin our discussion of the death process. Signs of death in the total body are familiar, including total body flaccidity with all muscles relaxed, absence of breathing and pulse, dilation of pupils and skin that blanches or becomes purple. A sequence of other timed changes occurs with the passing of time, which shows both interindividual and environment-associated effects. These include a range of diagnostic death signs, including pallor mortis (paleness due to lack of capillary circulation), algor mortis (postmortem cooling), rigor mortis (death stiffness), livor mortis (also known as postmortem hypostasis), and odor mortis (death smell – a significant sign of the commencing putrefactive changes) (cf. [121]).

24.3 Soft Tissue Change in Skin and Hair Follicle

One of the most important changes upon death is the cooling of the body's tissues. Maintenance of a stable internal temperature is required to keep enzymes functioning properly in cells and tissues so that they can drive the numerous metabolic processes essential for life [110]. After death, the body's temperature-regulating systems are disrupted, and heat is lost from the body and its associated tissues. Enzyme activity begins to diminish with reduction in temperature—typically if indoors the body loses approximately 1°C/h (excluding the first hour) and reaches room temperature only after 18–24 h, dependent on ambient conditions and the condition of the corpse (cf. [110, 112]). Although some metabolic changes can occur after death, these struggle to go to completion in the absence of oxygen. Here, formation of the so-called "molecular unit of currency" of intracellular energy transfer – adenosine triphosphate – from carbohydrate/glucose is impeded [80]. Instead, this reaction is stopped at the lactic-acid stage, which builds up within cells, causing loss of several ions (e.g., potassium and hydrogen) from the cells. Moreover, lipoprotein bonds in cell membranes, among others, break further, aiding tissue decomposition. Not all cells are sensitive to ATP depletion. For example, while the mesenchymal fibroblasts of the skin's dermis may be isolated and cultured *in vitro* from cadavers of up to 48 h post-mortem, the neural-crest derived melanocyte retains viability for less than half this time [111].

During life of the organism, there is some capacity for cells to adapt (ir)reversibly to injury (via hypertrophy, hyperplasia, atrophy, and metaplasia, hydropic swelling, and fatty change) [77]. However, when an organism dies, cells react to this cataclysmic "injurious" event by being overwhelmed and so they die.

Cell degeneration after the death of the organism is termed autolysis, whereas cells that die before the death of the organism undergo generally either a process termed apoptosis (programed cell death) or a process called necrosis (resulting instead from the inflammatory responses to acute tissue injury) [73]. The tissues are lyzed by the body's own enzymes and is not associated with inflammation. Autolysis is essentially therefore the self-rotting of the tissues at the cellular level. Still, it can be very difficult to distinguish early autolysis from for example, early coagulative necrosis due to ischemia.

The rate of tissue degradation can be influenced by multiple and highly variable environmental and other factors, including temperature, bacterial activity, and degree of exposure (cf. [121]). For example, the rate of cellular autolysis and of subsequent bacterial growth which defines putrefactive change will be significantly

reduced at colder temperatures. Similarly, the rapid desiccation of tissue due either to airflow or moisture loss in arid conditions will halt microbially-driven putrefactive change in its tracks, although tissue will only undergo these steps that lead to survival if entomology is limited or excluded from the outset (see below).

The hair fiber is formed within one of the most metabolically active tissues of the body called the hair follicle [104, 117]. Thus, it can be envisaged that a significant investment of energy is required to maintain hair fiber formation at anything near its optimal growth rate (0.33 mm/day in Caucasoid type hair and slower for africoid type hair [131]). It is no surprise, therefore, that the terminal hairs of the scalp are well supplied with nutrition via a rich vasculature. Its location in the body's largest and most exposed organ, the skin, also renders it very vulnerable to the insults of the external and internal environments [117]. However, despite the destructive changes that occur to the hair follicle and soft tissues after death, its product, the hair fiber, is a remarkably robust structure. One only has to look at how the hair fiber grows to appreciate how this structure is designed to last and last.

24.4 Hair Fiber Biogenesis

Hair follicles together with mammary glands mark us out as mammals. Approximately five million hair follicles reside in our skin, although only a paltry 2% are on our heads. There is much potency in the hair "signal" for humans, and so humans have spent considerable time and effort trying to change its form for sociologic and cultural reasons [117]. The hair follicle encapsulates all the important physiologic processes found in the human body, namely, controlled cell growth/death, interactions between cells of different histologic type, cell differentiation and migration, and hormone responsitivity. This hair follicle miniorgan deserves our further admiration for its ability to intersect with the body's systemic regulatory networks, aided by its own rich vasculature and innervations [104]. Remarkably, the hair follicle can respond to most hormones known to biomedicine. Even more surprising is the hair follicle's capacity to produce for itself a wide range of hormones, e.g., sex steroid hormones, proopiomelanocortin peptides, corticotrophin-releasing factor, and prolactin [109]. Further, neuropeptides, neurotransmitters, and neurohormones are implicated in mediating hair follicle events, particularly those related to stress [118].

Further kudos can be assigned to the hair follicle as our body's only permanently regenerating organ, as it transits through life-long periods of growth (anagen), regression (catagen), and relative quiescence (telogen) [117]. Each of these stages of the hair growth cycle is variably affected by microenvironmental and systemic changes. The observation that men castrated before puberty do not go bald nor grow beards, but did so after treatment with so-called male hormone testosterone, indicated a role of androgens in hair growth [117]. Lengthening the anagen phase will lead to larger, longer, and more pigmented hairs, while the reverse will also be true. Crucially, hair follicles in different regions of the body respond differently to different androgens.

The precise nature of the "clock" controlling hair cycling has been a long enduring enigma of dermatologic research. Many hypotheses have attempted to explain this, although none can yet fully explain all aspects of the hair growth cycle. Briefly, at the end of resting phase (telogen), a unique group of epithelial cells ("stem cells") in the upper hair follicle "bulge" are activated most likely by the adjacent follicular papilla cells. This stimulation is followed by stem-cell proliferation and their progeny go on to reform the lower "temporary" part of the hair follicle in anagen. Unlike the stem cell, proliferating "transit amplifying" cells have limited mitotic (dividing) potential before undergoing differentiation.

Cutaneous scientists have more recently only begun to appreciate the hair follicle's unique immunological status [25]. Unlike the rest of the skin, the lower portion of growing hair follicles is "immunosilent" because of its lacking tissue histocompatibility antigens. While hair canals contain a resident microflora of bacteria, including *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Demodex follicularum*, and *Malassezia* sp., the hair follicle appears to have a very effective antiinfection capacity, as evidenced by the rarity of folliculitis in human scalp, despite its 100,000 or so individual hair follicles.

While organismal death will have catastrophic implications for the skin and hair follicle preservation under normal environmental status, the hair fiber is much more resilient—although not indestructible. From an anatomic point of view, all hairs, irrespective of their caliber (diameter), length, color, or stage of

growth, are constructed of a bulk cortex surrounded by a protective covering of flattened cuticle cells [99, 117]. However, a third nonessential component, the medulla, is commonly located in the center, especially of large terminal hair fibers. The latter appears to be absent, intermittent, or complete depending on the body site of the hair (beard hair is usually medullated). This remarkable central cavity can appear with age and with pigment loss during canities.

The hair fiber is a highly integrated system of several components including in order of decreasing amount: "hard" keratins, water, lipids, pigment, and trace elements. The biosynthesis of hair proteins begins in the bulb of the growing (anagen) hair follicle, and for scalp, terminal hair follicles ceases approximately 500 μm above the zone of maximal keratinocyte proliferation. This level is still well below the skin surface, indicating that the hair fiber formation is "completed" long before it "enters the real word" (Fig. 24.1). Low- and high-sulfur proteins are synthesized here, although the synthesis of the latter peaks later. Despite significant variability the in hair form between humans of different ethnicities, chemical composition of hair protein across the ethnic groups is very uniform. There are no significant differences in amino acid composition of hair of different ethnicities [40, 117].

The "hard" hair keratins (7–8 nm across) can be distinguished from epidermal "soft" keratins by their lack of extended glycine runs. Instead, "hard" hair keratins contain many cysteine residues (particularly at the N- and C-terminal domains) that enable them to form extensive disulfide bond crosslinking with other cysteine-rich proteins. Moreover, the dynamics of assembly of keratin intermediate filaments in epidermis and hair fibers is very different. Although these filaments disassemble during epidermal keratinocyte division, they are the product of nonviable keratin-producing cortical keratinocytes in the hair follicle. To form a rigid and resistant hair shaft, abundant cysteine residues of the hair keratins need to be extensively crosslinked by disulfide bonds. Keratin-associated proteins (KAPs) facilitate such crosslinking, and various analytical tools (e.g., solubilization, chromatography, electrophoresis, and amino acid sequencing) have revealed an increasingly complex group of proteins. At least, 23 KAP families are now known, although research from the last couple of years promises to reveal even greater complexity, with large clusters of novel human KAP genes now located [117].

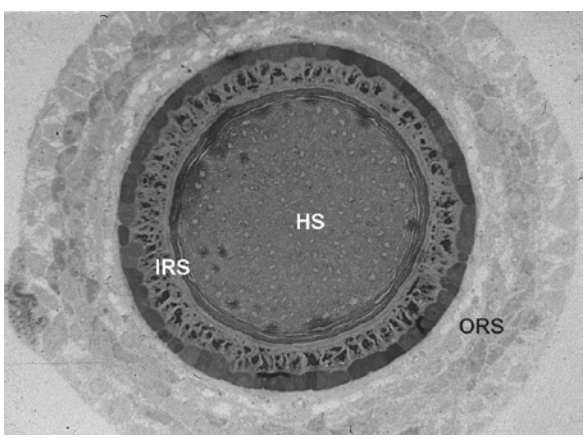

Fig. 24.1 Light microscopy view of transverse section of a human scalp hair follicle with central hair fiber (HF) surrounded by inner (IRS) and outer (ORS) root sheaths. Section was taken at the lower follicle level deep in the sub cutis of the skin, approximately 500 μm above the follicular papilla. Even at this level, the hair fiber is already highly compacted and differentiated

Lipids on the hair fiber surface (e.g., cuticle) provide a hydrophobic interface protecting the hair cortex from a hostile wet/dry environment [123]. It was not until the mid-1980s when a lipidic layer on the surface of hair fibers—termed the F-layer—was discovered, and this was found to contain a large amount (58% of total) of a methyl-branched 21-carbon fatty acid. The 21-carbon saturated fatty acid (exclusive to the hair/wool cuticle) is present in exceptionally high amounts and has been identified as 18-methyl-eicosanoic acid by mass spectroscopy. The main function of the branched methyl-eicosanoic acid is currently unknown, but it may be involved with increasing the degree of hydrophobicity over straight-chain fatty acids and/or altering the frictional quality of the fiber. In humans, the major fatty acids in hair fiber lipids include 16:0 (17%), 18:0 (10%), 18:1 (5%), and 21:0 (48%). It is of some considerable interest that hair cuticle lipids are highly conserved, in marked contrast to the high interspecies variability in sebaceous gland lipids. Evidence that lipids are surface bound is inferred from data showing that increasing hair fiber diameter is associated with a decrease in total bound fatty acid. Thus, it is now well appreciated that hair fibers have a 3–30 nm coating of long-chain fatty acids bonded covalently to the protein membrane of the epicuticle. Recent technologic advances, e.g., in atomic force microscopy, have allowed exceptionally high-power views of the hair

fiber surface and how this is altered by changes in temperature, hydration, pH, lipid layer removers, topically applied cosmetic products, etc. Small amounts of cholesterol sulfate, cholesterol, and fatty alcohol are also associated with the F-layer, although the nature of bonding (e.g., via thioester linkages) is unclear [123].

Human hair is commonly grouped into just three main subtypes: Caucasian, Mongoloid Asian, and African. Differences between these groups are usually determined with respect to a range of parameters, including hair fiber diameter and its cross-sectional form, overall fiber shape, mechanical properties (see above), combability, shape, pigmentation and pigment distribution, chemical make-up, and moisture level [99]. For many of these parameters, Caucasian hair falls intermediate to the Asian and African extremes. Recently, a systematic examination of the protein structure of hairs from Asian, Caucasian, and African individuals revealed no differences by X-ray analysis in the structure of the hair keratin. Asian and Caucasian hair fibers are more cylindric than those of Africans, and it has been shown that "breaking stress" and "breaking extension" values are lower in African hair fibers than in Caucasians or Asians. These features also aid identification for hair fibers found in forensic and archeologic contexts. Despite these gross ethnic differences in the form of hair fibers, the chemistry of the hair keratin is remarkably similar throughout all humans.

24.5 Survival and Recovery of Hair from Different Depositional Environments

The robust properties of hair as an external feature of mammals have ensured that it may persist in numerous types of depositional environment postmortem [131]. However, if a body is simply allowed to undergo putrefactive change, then the skin will start to blister and the epithelium is readily shed, taking with it the hair (see above) [60]. In such cases, the hair may then be dispersed by a variety of means dependent on the depositional conditions. For example, hair collected from surface-deposited remains may be used by birds and small mammals as nesting material [11], and in aqueous environments, the movement of water, combined with putrefactive changes, may result in total loss of hair. Another significant transformation in moist/wet environments is the formation of adipocere, a function largely of biochemical changes to subcutaneous fat [36–39]. In circumstances where adipocere forms, the skin will be disrupted by a variety of changes that include volumetric expansion and again the hair is less likely to be retained.

Archeology has shown us, however, that hair and fibers may persist over extended timescales [16, 132]. Perhaps, the most recognizable conditions in which hair may survive are those extreme environmental conditions in the archeological record that have resulted in exceptional preservation of other soft tissues – conditions that are conducive to natural mummification [7]. The term mummification has traditionally been reserved for desiccated remains, which may be recovered from cold-dry as well as hot-dry environments and with forensic casework can include temperate climatic conditions where there has been air-flow, such as within cellars or attic spaces [6], or where individuals were emaciated [59]. However, well-preserved remains may also be recovered from frozen, saline, calcareous/limed deposits, including cave sites [8, 44] or acid peat conditions where the action of decay microorganisms is inhibited [135]. In fact, some of the oldest known hair samples are nonhuman and derived from permafrost sites, and because of their condition, usable genetic information may still be recovered from these samples despite their age [48, 49] and alteration [47, 50].

It should be remembered that much of our current understanding of human hair is based on work first undertaken for the benefit of wool production and sheep breeding [55, 93, 124]. As such, it is important not to ignore the fact that keratin fibers from other species have relevance in archeology also. Much can be learned from fibers and the uses to which they have been put, and a combination of textile, environmental, and genetic evidence offers us an insight into the agendas of sheep domestication [23] and the trade and exchange of goods/animals/raw materials [2, 43, 74, 94, 119]. Human hair has also been put to use in the production of hats, textiles, mats, and even string used as bindings [1, 22].

Within Western Europe and North America, although unusual instances of hair and textile fibers are known from cist burials [32] and midden deposits such as found at Deer Park Farms in Northern Ireland [129], most archeological hair samples from temperate climes are derived from more recent eighteenth and nineteenth

Fig. 24.2 Plaited hair recovered from a 19th century burial in West Yorkshire, UK

century burials (Figs. 24.2 and 24.3). These contexts are varied in their preservation, particularly when you consider that these remains may be derived from both deep (sometimes waterlogged) urban deposits as well as crypts, and that coffin construction varies from wooden single shell to triple-shell, lead-lined coffins [95] and iron caskets [88], each of which has a contribution to the condition of hair and other organics held within.

Fig. 24.3 The differential preservation of hair shown in-situ during the excavation of a 19th century burial in West Yorkshire, UK

24.6 Significance of Hair in Forensic Investigation

The value of hair and fibers have long been recognized both in relation to forensic examination [96] and human identification [131], and various properties of hair aid in this regard – the large number of hair follicles on the average scalp; the mosaic hair cycle in humans ensuring loss of large numbers of hair fibers each day (approx. 150) via natural shedding; the frictional properties of the hair cuticle that provide contact evidence and allow hair and fibers to persist on clothing [90, 122]; the morphological characteristics (diameter, pigmentation, cuticle, cortex, and medulla form), which offer both species and racial distinction. However, hair shaft comparisons on purely morphological grounds [87] are rare, generally requiring the support of other scientific tools.

Key developments in the application of DNA-based techniques have seen the validation of mtDNA from the hair shaft in 1995 [136, 137] and the use of the recovery of low-copy number (touch) DNA from the hair bulb in shed or forcibly pulled fibers [54]. More recent work has investigated the fate of mitochondria in the keratinizing hair shaft [71], and the future potential of RNA from hair has also been proposed [12]. However, it is clear that hair is not without its difficulties when it comes to genetic analyses [52, 102, 131]. In cases of serious assault and murder, new approaches to the collection of hair and fibers such as total fiber taping [26] will see further interest in the evidential value of hair.

Hair is of critical importance in offering a perspective on chronic usage when it comes to the ingestion of either toxic or controlled substances. As a consequence, there is now a keen interest in the value of incremental data for instances of suspicious death, both with cases of long-term substance abuse [67] or questioned administration of prescription drugs [45, 84] and alcohol use [89, 91].

As an excretory tissue, hair is a natural sink for toxic materials and, as a consequence, is used in biomonitoring, with the potential to chart occupational exposure to heavy metals [30, 62]. Laser ablation inductively coupled plasma mass spectrometry even offers the potential to provide measurements on single hairs [107].

Stable light isotope data can provide important lifeways information for individuals [24]. Incremental stable light isotope measurements from hair have been used to

provide intelligence on the potential origin and movement of unknown individuals, as in dismembered and/or decomposed victim remains - discriminating on the basis of oxygen and hydrogen isotopes [35, 41, 42, 76, 82, 85, 92]. The same principle also offers the potential for intelligence on living individuals in the case of recent migrants [15] or terror suspects. Radiocarbon dating of hair and other tissues relative to global atmospheric values generated by nuclear testing since the 1950s has the potential to offer dating evidence for postmortem remains [83].

Increasingly, hair can have a major significance in the investigation of wildlife crime, and in this regard, protein sequencing and isotope analysis can be seen as two novel and highly discriminating tools to augment DNA investigation [101]. Considerable progress has been made toward sequencing of keratins for the purposes of species identification [57, 58], with progress made toward establishing reference data [56, 105, 139]. In this regard, it is important to remember that keratotics also encompass feather, hoof, horn, claws, and tortoise shell [3, 13, 28, 29, 31, 106, 140].

Hair itself is a physical trap for natural (soil, pollen, diatoms, testate amoebae, etc.) [138] and anthropogenic information (explosives and gun shot residue (GSR) data) [72, 141] both because of frictional characteristics of the cuticle and the fact that fibers are naturally imbued with a range of secretions from the apocrine and sebaceous glands.

24.7 Biogenic Information from Archeological Hair

Several sources have already flagged the unique importance of information from hair and fibers in archeology [127] covering various themes, including identification of fibers [108], dietary information and physiology, DNA, and drug use.

In archeology, the information derived from stable light isotopes, particularly when viewed as incremental data from hair segments, can have a bearing on key agendas, including diet and seasonality, physiological status, mortality patterns, social and economic status, trade and exchange, and locational information. Various studies are highlighted elsewhere [127]; subsequent isotopic studies have examined hair from curated samples of Plains Indians [100], from ancient Kerma remains from the Nile valley [112], and from eighteenth/nineteenth century deposits [130] and bog bodies [135]. One of the greatest prospects lies with segmental analysis of hair [5, 125], revealing for instance the mortality patterns from hair of Inca remains [126].

Several studies have examined in more detail the use of hair to offer lifeways information, including the remains of three children from Volcán Llullaillaco, North West Argentina providing evidence of status change in the 15-year-old Llullaillaco Maiden [133] and the Kwaday Dan Ts'inchi glacier mummy from North West British Columbia, who was found 80 km from the sea and whose bulk hair and bone cholesterol isotopic values indicate a shift in diet to include more terrestrial foods in the year before death [98], as well as groups of individuals from Peru [69, 125, 126].

New directions offering further potential with stable light isotope analyses include the ability to measure isotopic variation directly along single fibers using laser-ablation techniques [103] and development of the potential to analyze single amino acids from hair correlating these directly with dietary intake [75].

Based on the discovery that mtDNA can survive in fibers recovered from certain depositional environments [46], genetic information from hair has been used to address enduring questions such as the familial relationships of eight paleo-Eskimo remains discovered in the 1970s at the site of Qilakitsoq in Western Greenland [51]. Of these eight individuals, the relatively noninvasive nature of this testing (requiring in some instances only a portion of a single fiber) opened up for the first time the possibility of testing the 6-month-old infant from this assemblage, and thereby returning an identity and kinship to these remains that could not be afforded by other means. Further studies on material from the Duckworth collection have provided new insights into the origins and historical geography of certain mtDNA lineages of the aboriginal inhabitants of the Malay Peninsula [97], and more recently, nuclear DNA has been recovered from the hair of Siberian mummies dating from the sixteenth to early nineteenth centuries [4].

A pioneer in the application of drugs data from hair and other tissues from archeological remains is the research conducted by Larry Cartmell and coworkers. They provided the first direct evidence for use of coca in antiquity [19, 20] and have since examined the use of alcohol and other evidence from both South American and Egyptian remains [21]. Recent studies have sought to broaden the perspective of potential

drug records preserved in ancient hair to encompass other less well studied hallucinogens [9, 86].

The significance of hair in many cultures persists even today, with rites and rituals associated with the cutting of hair at distinct ages and a perception that hair is linked to witchcraft. When excavated, the Inca child mummies found on the tops of mountains in the South Central Andes were often accompanied by bags (made of either textile or animal intestines) filled with cut hair. In the case of the children from Volcán Llullaillaco in NW Argentina, mtDNA combined with isotopic evidence proved that these cut hairs were derived from the children themselves [133].

Concentration of toxic elements in hair [14] must be considered in the context of likely postdepositional uptake [64] or exposure to contaminants within historic collections. This is especially important in the context of quantitative data for prehistoric exposure to potentially toxic substances such as arsenic [63, 65, 66, 68] or methylmercury, which if real can provide valuable comparative data for current-day exposures, of particular importance for indigenous groups, for example, who continue to rely upon local traditional food resources [34].

Despite much literary and artistic evidence for ancient hair styles, hair stylistic information only rarely survives with physical remains. Recent scientific developments, examining both inorganic and organic evidence, have probed particular features of stylistic data from hair. Of note are hair preparations including the use of lead [120] and the recovery of cinnabar from the remains a high status Moche female individual from Northern Peru known as the Lady of Cao [78]; Paris team hair products; and the evidence of a "hair gel" and Celtic tonsure from the hair of the iron age remains of Clonycavan man at the National Museum of Ireland [79].

24.8 Taphonomic Considerations for Hair After Death

The term *taphonomy* is derived originally from the paleontological literature [33], but has been adopted more recently in both archeology and forensic science [113]. It is concerned with those influences (natural or anthropogenic – physical, chemical, or biological) and the resulting tissue transformations that occur from the immediate postmortem period through to their recovery and beyond. Consequently, it provides a key framework for understanding not only the survivability of material that may be looked for, but also the variables that will influence this. These influences on the survivability and decomposition of hair have been summarized elsewhere [128].

The relevance of taphonomy has been expanded to understand how different influences from the depositional environment affect the reliability of biogenic information, given that they may be altered or compromised by biological or chemical processes [61] and the influx of contaminants [53]. Similarly, it helps us to recognize the significance of appropriate handling, processing, and storage procedures from the point of discovery to the protocols for analysis, safeguarding against the risks of further alteration or contamination, and offering a key step in being able to assess the likelihood of spurious data. Although it has been demonstrated that with appropriate steps contaminating DNA can be removed to reveal authentic DNA even in degraded samples [47, 49], the sample processing methods can permit the successful removal of contaminants from even some of the most difficult of samples [119].

Many have observed the physical impact of keratinolytic organisms on hair, which in the case of fungi produce characteristic "tunnels" that bury into the hair shaft [27]. The external lesion that results from the penetration of the outer cuticle manifests as an ovoid hole with eroded margins (Fig. 24.4). Once the fungal hyphae

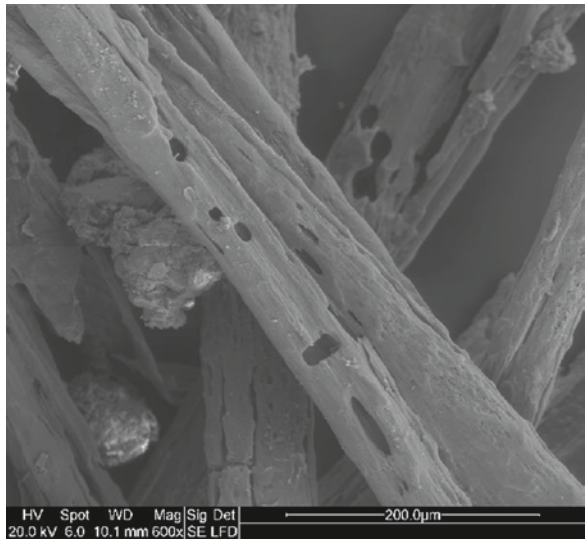

Fig. 24.4 Hair subject to fungal tunnelling by keratinolytic fungi

penetrate the cuticle, they can track laterally within the fiber, disrupting the cortex and medulla and affecting the different structures of the cortex in a predictable fashion [134]. The progressive destruction of the fiber, which ultimately results in the loss of keratin structures leaving melanin pigment granules and the remnants of cell wall structures, can be examined by means of a histological index [128]. These predictable changes can be correlated with the survivability of biomolecular information [47], although it is recognized that damage to degraded DNA templates may be highly specific in type, correlating with the geographic location and the taphonomic conditions of the depositional environment from which the remains are recovered [70].

It is important to recognize that, although there is a considerable interest in archeological remains, many such samples have languished in museum or private collections for many years, collections which in some instances may only have a partially documented history, raising questions of authenticity. This is exemplified by the varied origin of historic hair samples from different collections by means of mtDNA—samples that had all been attributed to Sir Isaac Newton [48].

Antiquarian interest, particularly during the nineteenth century, saw the assembling of numerous collections from many parts of globe, and these collections have generally only been subject to the scrutiny and protection offered by conservators and collections managers within recent decades. As such, hair samples from within these collections have often been subject to a variety of different insults from uncontrolled environmental conditions and exposure to both microbial and insect agents of decay; the indiscriminate use of biocides to try to limit infestations (including use of chemicals such as arsenic or nicotine); and to the unchecked habits of collectors and museum workers themselves in the vicinity of their collections (including smoking of tobacco and other products) [18, 81]. All such insults have made an impact on studies involving hair samples and, in some instances, have also spawned controversy, as with the findings reported by Balabanova and coworkers who claimed that nicotine, cannabis, and cocaine were present in samples of Egyptian mummy hair [10], now widely disputed [17]. This concern with the handling of hair and fibers is clearly recognized with modern forensic evidence and the collection and sampling of archeological fibers now overlaps with these ideals in terms of best practice.

24.9 Conclusions

Given the potential for hair to persist over both forensic and archeological timescales, it is hardly surprising that an expanding literature, highlighting the value of hair in these areas, now exists. It is now recognized that key properties of hair offer us the potential for unique data – the most significant being incremental hair growth, which enables segmental analysis to provide a diachronic picture that allows for subtle changes in the final months of an individual's life to be charted in great detail.

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