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# GENETICS OF HAIR AND SKIN COLOR

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■ **Abstract** Differences in skin and hair color are principally genetically determined and are due to variation in the amount, type, and packaging of melanin polymers produced by melanocytes secreted into keratinocytes. Pigmentary phenotype is genetically complex and at a physiological level complicated. Genes determining a number of rare Mendelian disorders of pigmentation such as albinism have been identified, but only one gene, the melanocortin 1 receptor (*MCR1*), has so far been identified to explain variation in the normal population such as that leading to red hair, freckling, and sun-sensitivity. Genotype-phenotype relations of the *MC1R* are reviewed, as well as methods to improve the phenotypic assessment of human pigmentary status. It is argued that given advances in model systems, increases in technical facility, and the lower cost of genotype assessment, the lack of standardized phenotype assessment is now a major limit on advance.



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## INTRODUCTION

Variation in skin pigmentation–skin and hair color—between people of different genetic ancestries is one of the most striking human characteristics (84). Study, and selection, of animals with particular pigmentary phenotypes has been of economic importance (5, 54, 55); pigmentation in the mouse and birds are classical experimental systems to study gene action (5, 54, 55, 104); and at the same time, even among nonexperts, it is widely understood that human skin color and hair color are largely under genetic control, reflecting a person's genetic heritage (97). One would have expected, therefore, the study of the genetics of skin and hair color in man to be a subject of much study: it isn't. For instance, we remain almost completely ignorant of such simple issues as the mode of inheritance of blonde hair. Indeed, although textbooks frequently refer to hair or eye color as an example to illustrate the role of genetics in understanding human diversity of form, until recently little was known of the genetic mechanisms underpinning normal variation in skin and hair color (14, 97).

Over the past ten years this situation has begun to change (5, 54, 56, 93, 106). Advances based on the asset of the mouse fancy (5, 55, 104), coupled with the facility of modern molecular technology, have allowed the identification of a number of genes important in the determination of skin and hair color in man. The genetics of many Mendelian disorders of medical importance such as albinism (63) have become clearer: Existing clinical classifications have been shown to be inadequate, and mechanistic likenesses between what were once thought to be distinct processes outlined (63). This review briefly discusses these conditions, but takes as its focus advances in our understanding of pigmentary variation within what may be arbitrarily, but usefully, defined as the normal population, describing in some detail the role of the melanocortin 1 receptor in human pigmentation (*MC1R*)—the only gene identified to date that appears to underpin variation in the normal population (93).

The review is structured into four parts. The first outlines the biology of human pigmentation, highlighting methodological issues in the assessment of pigmentary phenotype. The emphasis is on the assessment of phenotype and presentation of an outline of how complicated (rather than necessarily complex) phenotype can be. In the second section, the major Mendelian disorders of pigmentation—chiefly albinism—are briefly summarized. The third section deals in some detail with the melanocortin 1 receptor (*MC1R*), its genetics and molecular physiology, and what we know of the relation between *MC1R* genotype and human phenotype. Finally, I discuss areas that need to be developed and explored further, paying particular attention to the need to develop appropriate assay systems to understand the genetics of human pigment diversity.

## BIOLOGY OF HUMAN PIGMENTATION

Skin color is, except in rare pathological instances, the result of three pigments or chromophores: melanin, a brown/black or red/yellow polymer produced by melanocytes; hemoglobin in red blood cells in the superficial vasculature; and third, and to a much lesser degree, dietary carotenoids, sometimes most evident as a yellow color on the palms (90). Systematic differences in skin and hair color worldwide are principally the result of differences in the melanin content of skin.[1]

### Melanocytes and Melanogenesis

Melanin is a complex quinone/indole-quinone-derived mixture of biopolymers produced in melanocytes from tyrosine (51, 52, 88). Melanocytes are dendritic neural crest-derived cells that migrate into epidermis in the first trimester. Melanin production is associated with the production of a number of toxic intermediaries and largely takes place within a lysosomal-like granule, the melanosome. Melanosomes are secreted via a poorly characterized process into adjacent keratinocytes. Unlike iris melanocytes, epidermal melanocytes are therefore said to be incontinent, i.e., they secrete their melanin. Melanin chemistry is complex and remains poorly understood for a number of reasons that make chemical characterization difficult: It is a mixture of polymers; many intermediates are unstable and rapidly autooxidize; methods to solubilize melanin alter its primary structure (51, 88).

Hair and epidermal pigmentation are, in so far as melanocytes are concerned, similar processes: in interfollicular skin, pigment is passed from the melanocytes to the adjacent keratinocytes; in hair, a similar process exists, with pigment being added to the growing keratinocytes that will make up the shaft of the hair (hair is just one form of epidermis). In interfollicular skin, the melanocytes are found within the epidermal compartment immediately adjacent or close to the basement membrane. Melanosomes passed to adjacent keratinocytes tend to produce caps over the nuclei, shielding the nuclear material from ultraviolet radiation (UVR). This melanin is most evident in the basal compartment, the site of the keratinocyte proliferative compartment, but melanin remains with keratinocytes as they differentiate and move upwards—this melanin will still exert photoprotective activity on the cells beneath it (by casting a UVR shadow). In hair, the majority of what is visible to the eye is a dead structure, the color is the result of melanocytes in the hair bulb

[1]Abbreviations: $\alpha$MSH, $\alpha$-melanocyte stimulating hormone; ACTH, adrenocorticotrophic hormone; AHP, aminohydroxyphenylalanine; BAC, bacterial artificial chromosome; cAMP, cyclic adenosine monophosphate; CIE, Commission Internationale de L'Eclairage; DHICA-melanin, 5,6-dihydroxyindole-2-carboxylic acid; DOPA,3,4,dihydroxyphenylalanine; HPLC, high performance liquid chromatography; MC1R, melanocortin 1 receptor; MIM, Mendelian Inheritance in Man; OCA, occulo cutaneous albinism; POMC, pro-opiomelanocortin; PTCA, pyrrole-2,3,5-tricarboxylic acid; TYRP1, tyrosinase related protein 1; UVR, ultraviolet radiation.

passing their melanin to the adjacent keratinocytes as they undergo high rates of proliferation, stream pass the melanocytes, and then cornify (84, 90).

## Types of Melanin

Although the nature of melanin has frustrated precise chemical description, genetic approaches coupled with a number of available chemical assays have allowed some useful insights (5, 93). Melanin, with particular relevance to the current context, is commonly described as being of two principal classes: eumelanin, which is brown or black, and pheomelanin, which results from the incorporation of cysteine, is yellow or red [for reviews see (51, 80, 88, 89, 124, 125)]. One simple classification or description of pigment status is to consider two axes of melanin production: the amount of melanin(s) produced, and the relative amounts of either eumelanin or pheomelanin. The absence or relative absence of both melanin types is associated with white hair; a preponderance of eumelanin, with brown or black hair; and a preponderance of pheomelanin with red or yellow hair. A more precise chemical characterisation of the brown melanin polymers is possible (89).

Finally, color is not merely the result of the chemical composition of the various melanin polymers. Melanin is packaged into melanosomes, and melanosomes vary in shape and size (90). Such differences, by way of light scattering, will influence color, a fact colorfully illustrated by the way amphibians and fish disperse their melanosomes to influence their skin's color characteristics (3).

## Body Site and Temporal Variation

Differences in pigmentation between people are largely the result of differences in the amount and types of melanin produced, and the macromolecular structure and packaging of melanin, and not the number of melanocytes. There are, however, differences in pigmentation between different regions of the body as well as differences between people in the respective color of their hair and (interfollicular) skin (90). For instance, people from Northern Europe, such as many Scandinavians, have light or blonde hair and pale skin but their skin pigment increases in response to UVR to a moderate degree (94). Conversely, many people from Western Europe, such as the Irish, have pale skin, red hair, and a skin that tans little in response to repeated UVR irradiation. In the (relatively) unexposed body sites, such as the buttock, their skin may be similarly colored to that of Scandinavians, but in response to UVR it tans less, and the hair color may well be different—red as compared with blonde (94). Similarly, the color of some Caucasian skin may be similar to that of some Asians and yet the latter appears to have a greater propensity to tan in response to UVR or develop pigmentation after other inflammatory insults (such as from skin disease).

Furthermore, any idea of a unitary pigmentary phenotype has to take into account not only that skin color may vary between different body regions, but also that hair color may vary both in time and site. Scalp hair may be blonde in childhood and become brown or black in adolescence, before becoming white again in middle or old age. Beard or pubic hair may be red, and the scalp hair black or dark

brown. Skin color on sun-protected sites such as the buttock will be paler than on exposed sites but, even in those not exposed to UVR (such as the newborn), areas of skin such as the outside of the arms are a darker color than the inside.

A number of influences are at work to explain this diversity. First, in most individuals, repeated exposure to UVR causes an increase in facultative melanin production and possibly an increase in melanocyte number (36). Second, different body sites are preprogrammed to have differing numbers of melanocytes and constitutive melanin production (90). Third, the amounts and type of melanin production vary with age and by site, with children being paler skinned than adults, and females paler than males. Fourth, hair and skin melanocytes may show some degree of independence, that is the skin may be highly pigmented, the hair less so, although even this depends on body site. Finally, with age, melanocyte activity in hair, although apparently not in skin, may diminish, leading initially to a mixture of white and darker hairs (gray is a misnomer, as hairs are either white, or brown/black, rather than gray). The mixture of hair that is called gray may, under pathological conditions, revert to brown or black. The genetic factors responsible for virtually all these variations are poorly understood, if at all, and place important constraints on the precision of our current models of understanding of normal pigmentary variation. In general, they would be expected to lead to an underestimate of the strength of genotype-phenotype correlations in the absence of appropriate designs.



## Functions of Melanin

Many theories have been advanced for the biological role played by melanin (45). In other species pigmentation may either play an important role in avoiding attention by predators (camouflage) or, conversely, in drawing attention to particular biological forms [such as the blue scrotum of the vervet monkey, which appears blue because of light scattering of long-wavelength light from deep-seated "brown" melanin (87)]. In man, melanin plays particular roles in the eye and ear, but in the integument its roles are limited to (*a*) photoprotection against ultraviolet radiation and (*b*) sociocultural.

These functions are illustrated by the phenotype of albinos living in areas with high ambient UVR, such as Tanzania: Such individuals burn in the sun with pain, blistering, and an increased risk of infection and fluid loss; develop signs of premature aging of the skin (in reality, signs of excess UVR damage for their chronological age); and develop a range of skin cancers that may kill them as teenagers or in early adulthood (73). Melanin is an extremely effective sunblock, protecting against the harmful effects of electromagnetic radiation above $\sim$300 nm (98) (shorter wavelengths fail to pass through the atmosphere). UVR absorption, and hence protection, by melanin is greatest at the shorter wavelengths, where damage to nucleic acids and protein is maximal, and declines as a function of wavelength well into the visible spectrum ($>$400 nm). Although experimental studies are lacking, and therefore evidence is limited, differences in pigmentation, with differences in eumelanin of two- to threefold, can account for up to a 100-fold variation in sensitivity measured as the propensity to develop erythema in response to UVR

(2). The action spectrum, or propensity for UVR of any particular wavelength to cause erythema, is strikingly similar to that of DNA, suggesting that the former may be a useful proxy for the latter (27, 115). By contrast, ambient UVR varies less so, with total ambient UVR being ~3.5 times higher in equatorial regions than in areas such as Scotland (26). These total ambient UVR levels may be misleading in that a major determinant of individual dose will be ambient temperature in that in colder climates protective clothing as well as providing insulation will shield from UVR (25). The additional heat stress of a dark skin (black object) may also be relevant to any evolutionary tradeoffs.

For completion, various theories have been advanced to explain the lightening of skin as distance from the equator increases. The most popular is that since vitamin D is partly biosynthesized by keratinocytes in response to UVR (in the absence of abundant dietary sources), dark skin may lead to vitamin D deficiency in Northern climates (9, 53, 59). Rickets, with disastrous effects on reproductive fitness, would be one result, but other roles for vitamin D in protection against infection have also been posited as relevant (47, 53). Other explanations have also been suggested (19).



## Defining Pigmentary Phenotypes

It might be thought straightforward to measure skin or hair color in a biologically meaningful way (38, 94). In reality, the various disciplines concerned with skin color such as anthropologists, dermatologists, and cosmetic scientists have tended to develop their own methodologies. From the perspective of genetics, whereas color may be one realistic endpoint by which for instance assortive mating may be mediated (24, 119), resistance to UVR, albeit dependent on skin color, is not synonymous with it. Thus, although the amount and type of melanin (as explained above) may be a major determinant of UVR susceptibility, other factors also play a role. Even skin that has no pigmentation can develop photoprotection, defined as a reduced biological response to a subsequent dose of UVR (65, 71). This is thought to be due to the development of epidermal thickening and possibly qualitative changes within the epidermal layer (65, 71). Second, skin color may be similar between individuals, but their response to UVR may vary several fold (32). Such differences may account for some of the low skin cancer rates seen in some Asians even though they do not seem very brown; however, alternative and more mundane explanations such as behavioral differences may be more likely (18). Skin color is a key determinant of UVR sensitivity, but not the only one, nor should we equate color completely with our current understanding of melanin structure. The evolutionary determinants of skin color and resistance to UVR may overlap a great deal but they are not completely synonymous.

SKIN AND HAIR COLOR Skin color has been assessed in a number of ways. Broad-band spectrophotometry allows the sampling of reflectance (strictly speaking re-mittance) at a number of wavelengths such that wavelength-dependent curves

can be plotted (66, 103, 122, 123). How to manipulate these readings may not be straightforward, although most authors choose ratios or absolute values of one or more readings (70). Dermatologists have tended to use the tristimulus systems whereby color is represented according to one of a number of CIE indexes such as the L*a*b* score, where color is defined on three axes, light/dark, red/green, and yellow/blue (103). This color system is easy to manipulate and can be computed from broadband spectrophotometer readings, although there is a tendency to imagine that individual aspects of the index represent distinct biological qualities (e.g., melanin or blood), which may not be the case (15). Simpler instruments based on a few reference points using laser irradiation have also been used (28, 32). Any such approaches have to take notice of site, age, and sex variation, and changes due to ambient UVR. For instance, there are seasonal changes in virtually all body sites although they are least for the buttock (72). Second, it is questionable whether differences in erythema can be separated from those due to melanin because although distinct, the spectra overlap (66).

Hair color too can be assessed colorimetrically, as described above. Added pigments (hair dyes) will invalidate readings. Hair color charts have also been used but have obvious drawbacks in that ambient lighting is not controlled for, and they are subjective (31). The hair samples used are usually not natural, and match some populations better than others. As for skin, seasonal variation, although in this case due to bleaching caused by sunlight, is also a confounder.

CHEMICAL MELANIN ASSAYS The major classes of melanin, eumelanins and pheomelanins, can be assayed based on chemical degradation methods and HPLC analysis of specific degradation products using UVR and electrochemical detectors (51, 52, 124). Eumelanin is degraded into pyrrole-2,3,5-tricarboxylic acid (PTCA) by potassium permanganate oxidation of chiefly 5,6-dihydroxyindole-2-carboxylic acid melanin (DHICA melanin). Reductive hydrolysis of pheomelanin with hydriodic acid splits the sulphur-carbon bonds in pheomelanin to give rise to aminohydroxyphenylalanine (AHP) isomers. There have been relatively few studies in man of skin melanins and even systematic studies of hair are lacking (48, 85, 113). Dyed hair may interfere with the assays. To obtain appropriate samples of skin, biopsy is necessary. The assays are thus invasive and time-consuming.

SKIN SENSITIVITY TO UVR Another approach to defining pigmentary phenotype is to assay the skin's response to ultraviolet radiation in an experimental setting, using erythema as an endpoint (32). This is not as invasive as biopsy, is obviously dependent on skin melanin content, but also may reflect other factors including differences in inflammatory responses and in mechanisms for skin repair between persons. As changes in both pigmentation and blood flow may occur, reflectance instruments are invalidated except if used early following response (28, 29), necessitating use of a Doppler flow instrument or alternative methods to assess blood flow.

EPIDERMAL TRANSMISSION Finally, if biopsy is feasible, direct spectrophotometric examination of epidermis can be carried out (12, 13, 46). This has the possibility to dissociate pigmentary and nonpigmentary elements of photoprotection and provide more direct measures of biological dose than that obtained from reflectance.

PHOTOTYPE Originally introduced to allow better dosing of patients receiving photochemotherapy, this, at best, ordinal scale has found widespread use in clinical dermatology (30). It is based on the answers to questions about whether a person burns or tans in response to natural sunshine and to what degree. As a valid instrument it is subject to a number of limitations but is used widely (91).

Few studies have made use of the full range of technologies to define pigmentary phenotypes or to compare the various methods appropriately (94).



## ALBINISM AND RELATED DISORDERS

Albinism is one of the archetypal inborn errors of metabolism described by Archibald Garrod, with a frequency of around 1:20,000. It is usually defined as a congenital hypopigmentation of the skin, hair, or eyes (63). Ocular manifestations include reduced retinal pigmentation, abnormal decussation of the optic tract, nystagmus, and translucent irises (and hence reduced visual acuity) (63).

There are a number of different forms of albinism but all have in common a normal number of melanocytes but an impairment (to varying degrees) of the production of melanin. Some of the major forms of albinism are described briefly below but more authoritative reviews are recommended for a more detailed treatment (63).

### Oculocutaneous Albinism Type 1 (OCA1, MIM 203100)

This is the second most common form of albinism and usually produces a striking phenotype (63). It is due to mutations in the copper-containing enzyme tyrosinase, the rate-limiting enzyme in the hydroxylation of tyrosine to DOPA (3,4,dihydoxyphenylalanine) and dopaquinone. OCA1 is divided into two groups based on the phenotype, with OCA1 being more severe than OCA1B. Classically, the former patients show a complete absence of melanin in hair or skin at birth, with failure to tan or develop pigmented nevi. OCA1B may show freckles (a focal overproduction of melanin) and nevi (a focal increase in melanocyte numbers) and may tan a little in later life.

### Oculocutaneous Albinism Type 2 (OCA2, MIM 203200)

This disorder is due to mutations in the human homologue (*P*) of the pink-eye dilution mouse gene, and maps to 15q11.2-q12 (63). There are a number of phenotypes associated with mutations of this gene including that seen in most albinos in sub-Saharan Africa, with nevi, freckling, and lightly colored irises due to a large intragenic deletion (63). *P* may have relevance beyond this clinical group: *P*

mutation carriers have lighter skin than controls, and patients with Prader-Willi or Angelman syndromes, which may affect the *P* gene, have lighter skin than other unaffected family members (96, 106, 107).

### Oculocutaneous Albinism Type 3 (OCA3, MIM 203290)

At least in African populations this is characterized by reddish-brown skin, ginger hair, and brown irises. It is due to mutation of the tyrosinase-related protein 1 (*TYRP1*). Not all of the usual ocular manifestations of albinism may be present (76, 106, 107).

Other types of albinism and related disorders are reviewed by King (63). The example of OCA2 and OCA1B suggest, at least to this author, that sequence variation in some of these loci may underpin pigmentary variation in the normal population. It is worth contemplating that in individuals from pale-skinned populations, such as those in Northern or Western Europe, forme fruste phenotypes may be harder to recognize than in African populations.



## THE MELANOCORTIN 1 RECEPTOR (*MC1R*), (MIM 155555)

In 1993, Cone and co-workers identified the extension locus as the melanocortin 1 receptor (*Mc1r*) (95). A number of mouse mutations had previously been mapped to this locus, with recessive mutations producing yellow or pheomelanin hair, as compared with a brown/black eumelanic wild type (20, 104). Dominant gain-of-function mutations resulted in a black color due to increased eumelanin (104). The *Mc1r* encodes a seven-pass-transmembrane receptor that, when activated, signals via cAMP to increase the eumelanin/pheomelanin ratio (20). The receptor has been likened to a pigmentary switch: activation leading to brown or black melanin at the expense of yellow or red pheomelanin. In mouse the endogenous ligand is $\alpha$-melanocyte stimulating hormone ($\alpha$MSH), although other related peptides such as adrenocorticotrophic hormone (ACTH) also show activity at the receptor (20). In mouse there is a also a physiological antagonist, AGOUTI, produced in skin and acting in a paracrine manner to oppose the effects of $\alpha$MSH (79).

The human *MC1R* was cloned (17, 82) and is located at 16q24.3 (35). Cone presciently predicted that *MC1R* variation may underpin human pigmentary variation, mentioning red hair as an example (95). It is at this stage worth explaining the differing nature and color of *MC1R* mutations.

### Red Versus Yellow

In mouse, mutations reducing function at the *Mc1r* cause yellow hair with melanin assays showing a high phaeomelanic/eumelanic ratio (95). However, in other species and in man, the phenotype is different, with a red rather than yellow color being the dominant phenotype (93, 117). The situation in dogs is illustrative (83):

Red setters are—given the name, not surprisingly—red, but the same *MC1R* mutation in labradors is accompanied by a yellow color. The reason for this difference, presumably due to interaction with other loci, is not known.

In man, the *MC1R* encodes a predicted 317-amino acid protein (40). Like many G-coupled receptors, there are no introns within the *MC1R*, although claims have been made for another exon 5′ although (as yet) it appears of little physiological significance (37, 110). There are four other known receptors in the melanocortin family (20, 21, 82): the MC2R, better known as the ACTH receptor; the MC3R and MC4R, which are found particularly within the central nervous system, with the latter playing a key role in energy homeostasis; and the MC5R, which is widely expressed but in mouse plays a key role in exocrine gland function, particularly in the sebaceous glands of skin (and possibly in human) (16, 39). In terms of pharmacology, the various receptors show some similarity of response to ligands but the activity characteristics for the MC2R appear distinct from the others (20, 99–101), and there appear differences between mouse and human. ACTH, another cleavage product of pro-opiomelanocortin (POMC), is also active at the MC1R in human.

Initial sequencing of the *MC1R* in human showed that sequence diversity was common and that some changes seemed to be associated with people with red hair and pale skin (117). Before describing in more detail these sequence variants, other evidence is cited about the role of this signaling pathway in human pigmentary physiology.



## $\alpha$MSH and Human Pigmentation

Although $\alpha$MSH, a cleavage product of POMC, was named because of its pigmentary activity, and despite early experiments showing a role for exogenous $\alpha$MSH and the closely related peptide ACTH in increasing pigment formation (eumelanin) in human, there was still doubt about whether this pathway had any function in human (as compared with mouse) (69). In part, this was because circulating levels of these hormones were thought to be below physiological significance, but also because in some cell culture conditions, addition of melanocortins did not increase melanocyte pigmentation (34). On the other hand, other experimenters did see an effect of $\alpha$MSH in melanocyte cultures (23, 49) and $\alpha$MSH had been identified in skin previously (114), raising the possibility that local control was more important than circulating levels. This latter point only exemplifies the everyday observation that tanning in response to UVR, which is thought to be in part mediated by $\alpha$MSH, is a local rather than circulating effect. Another question relating to this signaling pathway was whether a ligand was physiologically necessary in human, or whether the receptor possessed intrinsic activity. However, subsequent to the reporting of associations between sequence diversity at the MC1R, and pigmentary phenotype in human (117), two siblings were reported from Germany, with bright red hair (in the absence of a family history) and a complex endocrine phenotype resulting from a mutation at the (*POMC*) gene (MIM 176830) (68). The sibs were therefore unable to produce ACTH or $\alpha$MSH, demonstrating that at least one of them is

necessary for activation at the MC1R. The relation between ligand and receptor may differ between mouse and human (4, 42).

## Genotype-Phenotype Correlation at the *MC1R*

Studies have shown that the *MC1R* coding region is highly polymorphic, with over 35 segregating sites identified to date (11, 37, 92, 105, 107, 117, 118). An initial (and remaining) problem was to define which alleles were functionally significant. No gain-of-function mutations have yet been identified although the status of the R163Q, common in Asian populations, has been poorly studied (11, 37, 92). No sequence variation outside the coding region has been shown to account for phenotypic variation in man (37; A.J. Ray & J.L.R., unpublished observations).

Human, family, population, and disease association studies have shown a number of repeatable phenotype associations with MC1R diversity. The R151C, R160W, and D294H changes are clearly associated with red hair and pale skin (11, 31, 41, 105). Most persons with red hair are either homozygous or compound heterozygous for a combination of these changes (11, 31, 105). Perhaps 10–20% of individuals with red hair show only a change on one allele, although these individuals tend to have lighter red-colored hair than those harboring two diminished function alleles (31) (strawberry blonde rather than carrot). If chimp sequence is treated as the root haplotype, some human alleles carry more than one change from consensus but only a single change on a singular-allele has been causally implicated in red hair (37). In a Northern UK population, over 40% of the population harbor known functionally significant alleles with diminished function such as the R151C or R160W (41).

To support a causal role of these specific changes in the phenotype, heterologous transfection assays with cAMP as a read out in response to $\alpha$MSH or similar ligand show diminished, but not absent, activity (33, 102). All these variants are able to bind the receptor (33, 102). Bacterial artificial chromosome (BAC) rescue of homozygous *Mc1r*$^{e}$ (null) mice has also been carried out to further define the nature of these sequence variants (42). In this assay, the different alleles were also not complete loss-of-function alleles and appeared to impair signaling to different degrees, with a greater impairment of function with D294H than with R151C or R160W (42).

Other studies have also highlighted the important role of these diminished function alleles. Thus, whereas all the human studies alluded to have classed hair color according to author-defined criteria, associations have also been seen with a range of other phenotypic characteristics. This includes a tendency to burn rather than to tan in response to repeated irradiation (based on subject recall) (41); objective assessment of freckling (6, 31, 105) and solar lentigines (6); and associations with both melanoma and nonmelanoma skin cancer (7, 10, 59, 61, 86, 105). Risk ratios have typically been in the range of 2–5, with clear evidence of a heterozygote effect.

The relation between *MC1R* sequence change and skin cancer has been the subject of different interpretations. What is not in dispute is that the alleles

mentioned above are associated with a variety of human skin cancers including basal cell carcinoma, squamous cell carcinoma, and melanoma. (The situation with respect to other alleles is discussed below.) The most immediate, and to this author most plausible, explanation is that since the *MC1R* is a determinant of pigmentary phenotype with wild-type function enhancing eumelanin rather than pheomelanin production, and since eumelanin is more protective than pheomelanin against the harmful effects of UVR, then it is only to be expected that associations between *MC1R* and skin cancers are seen. Since UVR is the major environmental cause of skin cancer, and pigmentation the major genetic determinant of UVR protection, it is no surprise to see such an association. Indeed, the available logistic regression equations show such an effect. However, in many studies, even when clinical measures of Fitzpatrick phototype (30) are taken into account, effects of the *MC1R* still persist. Two explanations have been advanced. The first, favored by this author, is that this simply reflects the inadequacy of the Fitzpatrick phototype as a measure of pigmentary status (94), flattening the regression fit and thereby allowing the effect of *MC1R* still to be seen. From this standpoint, what is seen is a methodological rather than a biological issue. An alternative viewpoint is suggested by others (61, 86). There is a body of literature ascribing effects to various melanotrophic peptides acting via the MC1R on melanocyte growth (rather than on melanogenesis) and on cells other than melanocytes such as endothelial cells or immunocompetent cells (8, 44, 74, 81, 108, 112). From this viewpoint, it can be considered that the *MC1R* exerts its effects not just through the amount and type of melanin protecting against UVR but via other nonpigmentary mechanisms. Since the relation is with tumors derived from different cell types, both of which are UVR induced, any common mechanism proposed cannot be cell-type specific (such as involving the melanocyte alone).



## Spectrum of Sequence Diversity at the MC1R

Initial association studies were confusing because of the degree of diversity at the *MC1R* (43, 50, 67, 117, 118), even though the absolute degree of diversity may not be very unusual (37). Thus in the initial publication, although some variants appeared associated with a red hair/pale skin phenotype, it was unclear which of the many alleles were significant, as only a minority of the population had the consensus sequence, and any mode of inheritance was unclear. Subsequent association and family studies and functional studies have, in part, resolved the role played by several alleles including the R151C, R160W, and D294H as described above (and some others, 57). There are, however, uncertainties remaining. For instance, the V60L allele is very common (15% of a UK population) (31), heterologous transfection assays show it to possess diminished signaling (102), and some association studies have shown associations with cancer phenotypes (7, 61); some family studies have also been reported modeling the V60L as a low-penetrant diminished-function allele (31). For some other alleles, such as the D84E, the functional status remains unclear, with discrepant findings (7, 43, 61, 118).

Reference was made earlier to the fact that in BAC rescue of *Mc1r*$^{e}$ mice, the R151C, R160W, and D294H alleles were not functionally equivalent (42). It may make sense therefore to think of a broad range of alleles possessing various degrees of activity, with the observed phenotype depending on other genetic (or other) influences as well. Because there are a large number of rarer alleles, current association studies have limited power to test associations, and functional studies on many alleles have not been carried out. Association studies that classify alleles as either wild-type (or pseudo wild-type) or functionally significant are likely to contain nonrandom classification errors. It also remains unclear how sensitive the in vitro assays are for variants with minor differences in activity.



## Role of Agouti in Man

In mouse, AGOUTI signaling protein, produced in a paracrine manner from the dermal papilla, antagonizes the effects of $\alpha$MSH at the MC1R (1, 4). Two studies have looked for a role of this protein in human by relating sequence diversity to pigmentary phenotype (60, 120). No nonsynonymous coding region diversity was found in either study, but a weak association was seen in the 3′ untranslated region with dark hair color in one study (60). Expression studies of agouti have not been published in man either, so resolving this issue will require larger studies, more precise estimates of phenotype, and a better understanding of the relation between sequence change and function around the agouti locus.

Some other indirect evidence about any putative role of agouti in human comes from the rescue of *Mc1r*$^{e}$ mice with human *MCIR* (but under a mouse promoter) (42). These experiments show that wild-type human MC1R is relatively resistant to the effects of AGOUTI and that mouse AGOUTI is still able to bind mutant human MC1R.

In some animals agouti is under the control of promoters with body site specificity giving, for instance, a lighter coat ventrally than dorsally (121). In human, there is also body site variation with red beards being present in some men with dark scalp hair. It is tempting to imagine analogous mechanisms with different agouti promoters being active in different body sites but there is no evidence as yet to support this speculation.

## Evolution at the MC1R

Mutation at the *MC1R* underpins integument color changes in a wide range of animals apart from human, including dog (83), fox (116), bird (109, 111), pig (62), horse (77), and cow (58, 64). Whereas in many species such changes reflect uses of pigment in camouflage or sexual behavior, or selection by human for particular phenotypes (such as in dog), pigment in man has largely taken on different functions. In many species, such as mouse, interfollicular skin has few, if any, epidermal melanocytes. Protection against UVR is provided by hair—of any color–except, for instance, on sites such as the ear of mice that have only scant hair, where melanocytes are not confined to the follicle but are found in the interfollicular

epidermis. In human, melanocytes are found in both follicular and interfollicular skin where they play a major role in protection against UVR. How is the variation in human skin and hair color to be explained?

Several studies have taken advantage of sequence diversity to answer this question (37, 75, 92). Although the data produced, based on sequencing of *MC1R* in diverse human populations, have many similarities, the conclusions are different (37, 75, 92). It is worth thinking through what questions can be asked of the data.

One possibility is that variation at the human *MC1R* is a result of selection. In turn, selection could be accounted for by the need to protect against burning from UVR in equatorial regions. By contrast, in areas with low ambient UVR, such as Northern Europe, the need to avoid rickets by maximizing the amount of UVR that reaches the keratinocytes that synthesize vitamin D could be another factor favoring loss of pigment (53). Other reasons for selection could include active choice of mate with particular phenotypes (24). An alternative view is that much variation at the MC1R is due to random change rather than to selection.

Published studies show that sequence diversity is greatest at the *MC1R* in non-African populations: European populations show much higher diversity than African or Asian populations (37, 92). Harding et al. (37), based on a study of 224 individuals from around the world, argue that given the diversity of sequence between chimp and human and the time of evolutionary divergence, the low rate of sequence diversity in African populations reflects functional constraint. By contrast, they argue for release of this constraint in European populations (they find no statistically significant evidence in their dataset for departure from neutral theory to explain European diversity). Using a coalescent model they can now date these mutations to around 20–40,000 years ago, earlier than their published estimates (37) (R. Harding, personal communication). Conversely, Rana et al. (92), based on a study of 121 individuals from around the world, argue, chiefly on the basis of the ratio of synonymous and nonsynonymous changes, for selection operating at the *MC1R* in European populations. What is not in doubt are the broad similarities between the datasets and the contrast to many other loci where diversity is greater in African populations. The robustness of the statistical models and tests need to be reviewed in the light of future studies. Although it does not help choose between the competing interpretations, the differences in pigmentary phenotype between the populations studied cannot be solely explained by changes at the *MC1R*. Red hair and blonde hair, the latter of which cannot be explained by changes at the *MC1R*, are both more common in Northern European populations, as is pale skin.



## FUTURE STUDIES AND THE DEFINITION OF PHENOTYPE

The original studies on red hair inheritance were published almost a century ago by Davenport (22). Red hair approximates to a recessive trait: the presence of two of a limited number of sequence variants in Northern European populations results in a

very high chance of having red hair (~0.95). The focus on the *MC1R* in this review reflects that it is the only gene known so far that plays a part in normal variation in pigmentation. There are several reasons for this. There was a powerful animal model; the gene was one of a known family of receptors whose pharmacology was well understood; sequence variants were confined to the coding region of a small intronless gene; and many variants at the locus had a large effect on phenotype.

Even so, statements that this particular allele causes red hair or pale skin are imprecise and inadequate. Where does red hair begin and end; how do we mechanistically think of the heterozygote effect on phototype and cancer risk; how much of the variation in human pigmentation can we attribute to this locus? Most of the studies described above (including those from the author's laboratory) are inadequate to answer these questions with the necessary precision: to take the subject further we will, among other things, require more appropriate quantitative techniques to document phenotype and allow comparison between different groups. Put simply, how can we critically review different studies when their authors provide no transferable definition of red (hair) or of pale skin? In the remainder of this review, I anticipate one way in which progress can be made, using early results from my own laboratory to illustrate the approach.

Early studies of the *MC1R* and red hair provided no definition of red beyond a simple operational definition used by the investigator (11, 117). Even so, a single (or composite?) group "red" emerged after bootstrapping back to *MC1R* sequence so that shades such as carrot red, strawberry blonde, and auburn were included together (31). A chart linking genotype with crude color assessment using this method and objective HPLC analysis of melanins is revealing (Figure 1): Based on hair melanin measures the "color" varies considerably and the sequence data lend credence to the idea that there is greater clustering of phenotype if melanin data are used rather than categorical classification of hair. Indeed, simple colorimetric measures may be useful as there will be a relation between melanins, hair color using spectrophotometry, and sequence (Figure 2). The advantage of such methods is objectivity and the ability to handle the trait quantitatively. The disadvantage is that rather than relying on recall of hair color at a certain age, subject age needs to be taken into account in the study design. The possible advantage of such an approach is illustrated by a recent report (78).



## Phenotype of MC1R Variants Against "Black Skin"

Observation would suggest (to this author) that the effects of the MC1R would vary on different genetic backgrounds: That this is so would hardly be surprising. How do we measure this effect? For instance, would we expect to see an effect of a single R151C, R160W, or D294H allele on a black African? Would we expect to see the same effect on hair and skin? We have recently, in collaboration with Colin McKenzie in Jamaica, studied three red-headed children born to self-described black parents (78). The children harbor some of the *MC1R* mutations seen in Northern Europeans. In photographs their hair color is obviously red, but

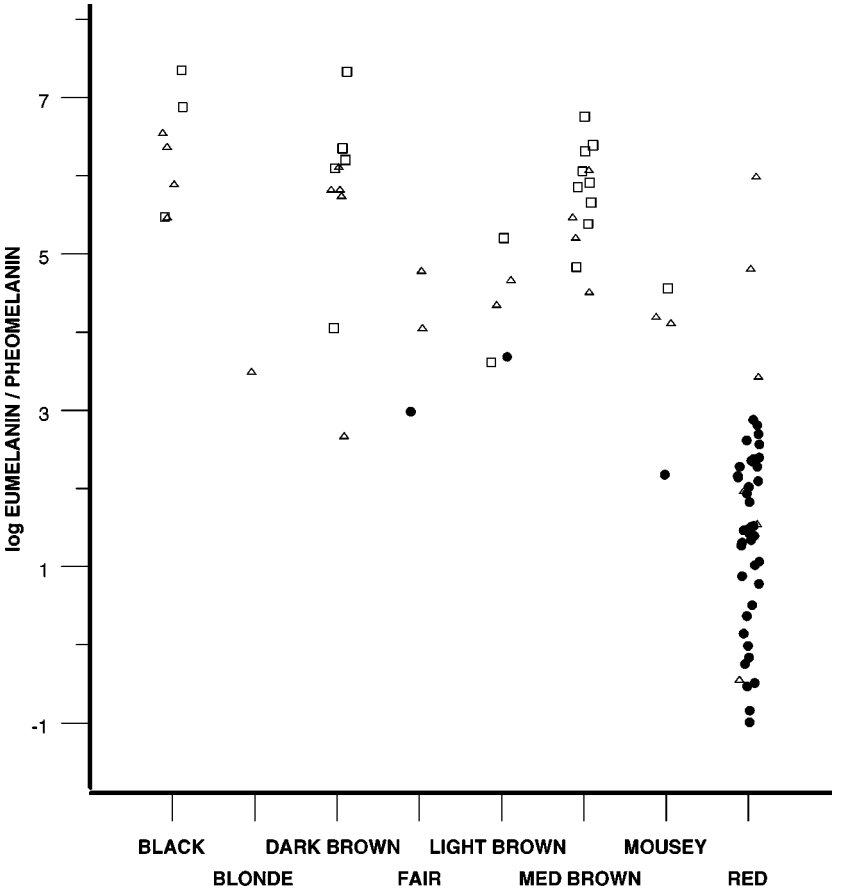


**Figure 1** Hair color classified using a hair chart against log of eumelanin and pheomelanin ratio by genotype (*closed circles*, homozygous diminished function; *triangles*, heterozygote; *open squares*, wild-type or pseudo wild-type).



more usefully, hair melanins were assessed quantitatively showing values within the range seen in persons with red hair from Northern Europe (J.L.R., unpublished). These results do not allow for differences in age, but at face value suggest that the homozygous effects of MC1R, even on a very different pigmentary background, are large and observable. Perhaps the effect is greater on hair than skin? One can imagine a similar experiment in European populations. Traditionally, there is a pocket of red-headed persons in Naples. How much difference would we expect between *MC1R* genotypically identical individuals between Naples and Edinburgh? When studied quantitatively, estimates of the relative contributions of this locus and that not due to this locus can be formulated. To carry out this plan requires standardized methods to assess hair color, agreed by different investigators,

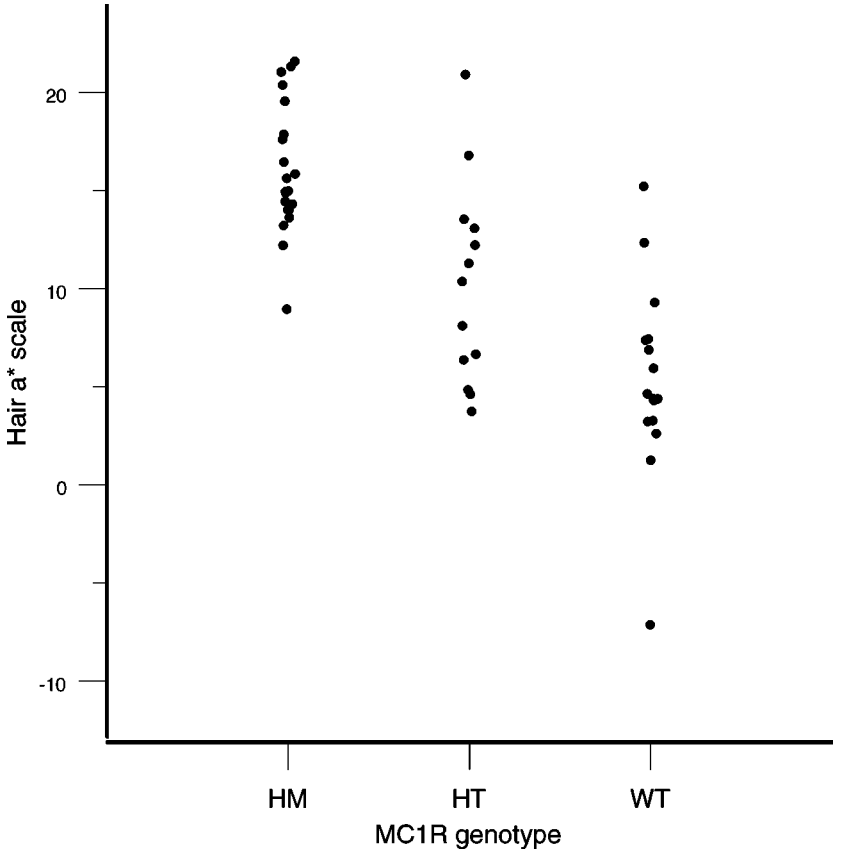


**Figure 2** MC1R genotype against hair a* color using the L*a*b* system where a* represents a polar co-ordinate on the green/red axis. HM, homozygote; HT, heterozygote; WT, wild-type or pseudo wild-type.



and simple descriptive epidemiology of pigmentary characteristics of various human populations.

The second issue for the future relates to genes other than the *MC1R* and the magnitude of effect searched for. The mouse and other model systems have identified a large number of genes important in determining coat color. To be detected using classical screens, their effects are likely to have been large and interpretable as Mendelian traits. Using these genes as candidates in human has until recently been hampered by the costs of sequencing and the lack of widespread acceptance of the need for more accurate formulation of phenotype. Both these impediments have now changed. Sequencing of a wide range of candidates looking for associations between coding or noncoding sequence variation and phenotype is now practical, and although effects in mouse and human for a particular locus may

not be comparable, the mouse allows subsequent functional assays to be planned. The experimental caution remains that without family studies or functional assays, the causality of any haplotype changes with pigmentation may be confounded by any relation between pigmentary characteristics and evolutionary ancestry.

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