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The Genetics of Human Skin and Hair Pigmentation

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Keywords

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Abstract

Human skin and hair color are visible traits that can vary dramatically within and across ethnic populations. The genetic makeup of these traits—including polymorphisms in the enzymes and signaling proteins involved in melanogenesis, and the vital role of ion transport mechanisms operating during the maturation and distribution of the melanosome—has provided new insights into the regulation of pigmentation. A large number of novel loci involved in the process have been recently discovered through four large-scale genome-wide association studies in Europeans, two large genetic studies of skin color in Africans, one study in Latin Americans, and functional testing in animal models. The responsible polymorphisms within these pigmentation genes appear at different population frequencies, can be used as ancestry-informative markers, and provide insight into the evolutionary selective forces that have acted to create this human diversity.

INTRODUCTION

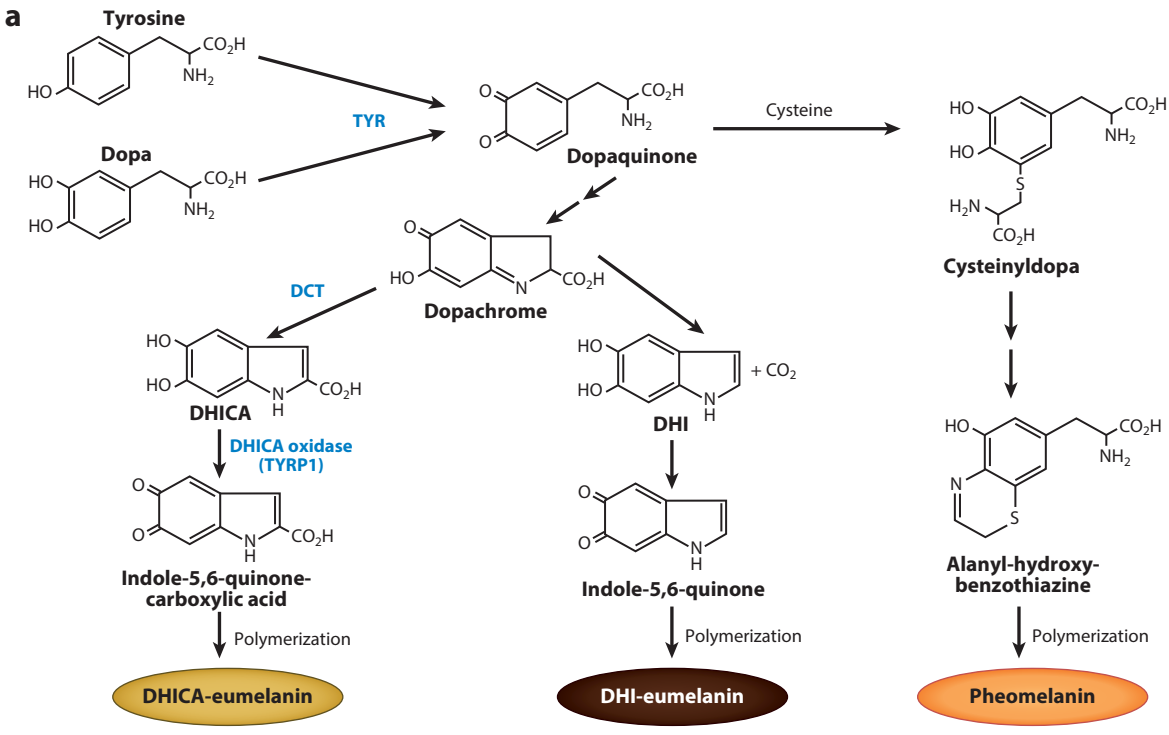
The abundance of melanin pigments and their chemical and physical properties largely determine the coloration of human skin and hair, along with other mammalian features, such as the color of fur. Our understanding of the genetics of melanin formation and distribution within cutaneous and follicular tissues has recently greatly expanded through the power of genome-wide association studies (GWASs) using large databases such as those of the UK Biobank (22) and 23andMe (51). This research has provided new insights into the biology of skin and hair color and underscores the highly polygenic nature of these two traits, with complex epistatic interactions apparent between the genes involved.

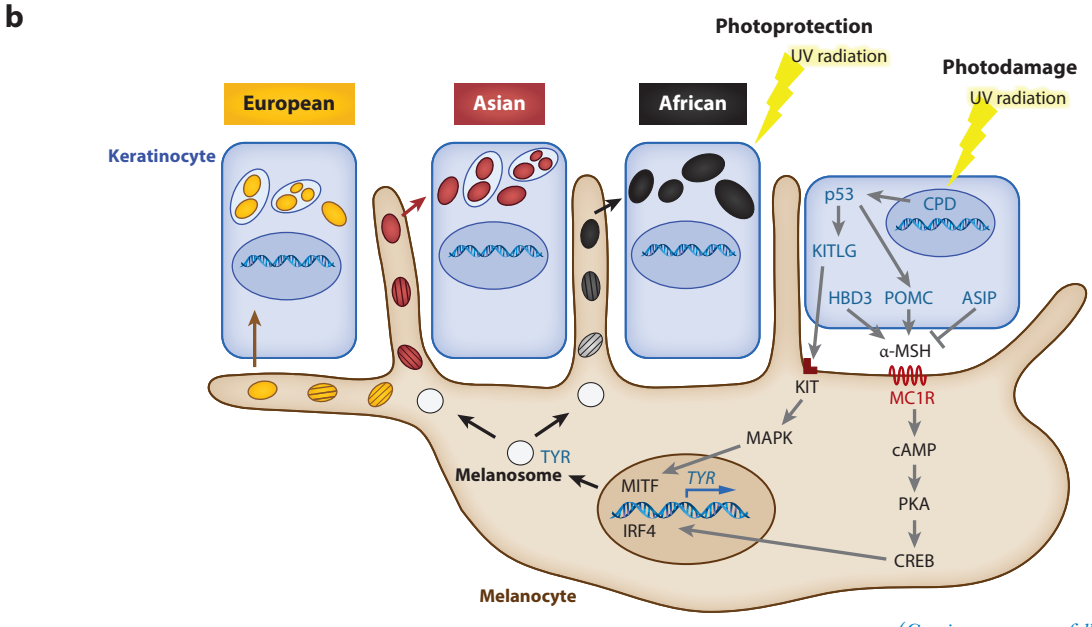
The geographical patterns of ethnic skin pigmentation show a strong correlation with incident UV light intensity, which has been a strong selective pressure (137). Apart from albinism conditions, most of our knowledge of the genetic landscape of human pigmentation has come from predominantly European populations. These studies have looked at genetic loci and polymorphisms that explain differences in pigmentation within European populations or between European and non-European populations. Only recently has a more global approach to understanding pigmentation gene variation in African (34, 100), Asian (48, 115, 159), South Asian (64, 74), and Latin American (5) populations been attempted.

Melanin is a macromolecular biopolymer that is derived from the oxidation and polymerization of tyrosine (35) (**Figure 1*a***), synthesized in epidermal melanocytes, and transferred to the surrounding keratinocytes (**Figure 1*b***), to help protect against the effects of UV radiation. Specialized melanocytic enzymes and structural proteins are trafficked and assembled into the melanosomal particle in a maturation process leading from an empty vacuole to a striated melanin-filled organelle, designated in four stages. This process includes the passaging of the key tyrosinase (TYR) enzyme, catalyzing the first step of the melanin biosynthetic pathway, which is the oxidation of its substrate tyrosine to form the intermediate dopaquinone.

Two types of melanin are produced within specific organelles called melanosomes: brown/black eumelanin and red/yellow pheomelanin. The ratio of eumelanin to pheomelanin depends on the catalytic activity of the rate-limiting melanogenic enzyme TYR and the availability of cysteine. The dopaquinone intermediate is much more reactive with cysteine, which favors the production of cysteinyldopa over intramolecular cyclization to form dopachrome via cyclodopa (66). In addition, several melanosomal ion transport proteins are required for melanosomal function (17), and the regulation of melanosomal pH is critical for the process of melanogenesis (**Figure 2*a***). Notably, melanosomes from melanocytes of fair-skinned individuals are more acidic and display low TYR activity, whereas melanosomes in dark skin are more neutral and present higher levels of TYR activity. In the casing model of mixed melanogenesis (154) that occurs within each melanocyte (35), a pheomelanic melanin is first produced as the default pathway, which upon cysteine depletion is followed by the deposition of eumelanic pigment around this core (**Figure 2*b***).

The process of melanocyte development and the biochemistry of melanogenesis are largely conserved across vertebrate species (32). Chemical characterization of mouse strains with different coat colors has allowed a good correlation between visual phenotype and the ratio of pheomelanin to eumelanin. The diversity of human hair colors has similarly been examined (65, 66); a small but constant amount of pheomelanin was seen in black, dark brown, medium brown, light brown, and blond hair, while the eumelanin content varied depending on the intensity of the color, with the highest for black and the lowest for blond. Only red hair contained substantial amounts of pheomelanin, and, consistent with mixed melanogenesis, a comparable level of eumelanin was also present. Testing of the melanin components of cultured melanocytes derived from diverse





(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

(*a*) Melanogenic pathway leading to eumelanin and pheomelanin. The process of melanin biogenesis begins with the oxidation of the substrates tyrosine or 3,4-dihydroxyphenylalanine (dopa) by the tyrosinase (TYR) enzyme to form dopaquinone. In the absence of cysteine, there is an intramolecular addition to produce the red-orange intermediate dopachrome, which either spontaneously decomposes to 5,6-dihydroxyindole (DHI) or, with the catalytic action of dopachrome tautomerase (DCT), produces 5,6-dihydroxyindole-2-carboxylic acid (DHICA). The DHICA-eumelanin polymer produced through the involvement of tyrosinase-related protein 1 (TYRP1) is a lighter color than the DHI-eumelanin polymer, which is dark brown or black. In the presence of cysteine, dopaquinone is conjugated to form cysteinyldopa, with further steps resulting in the production of the red/yellow pheomelanin polymer. (*b*) Keratinocyte–melanocyte interactions involved in the UV-induced tanning response and melanosomes in different skin types. The figure is a schematic representation of a dendritic melanocyte cell (*brown*) interacting with keratinocytes (*blue*) within the skin. The keratinocyte on the right is exposed to UV radiation that induces DNA photodamage, such as cyclobutane pyrimidine dimer (CPD) formation, which activates the p53 protein, leading to proopiomelanocortin (POMC) and KITLG production (signaling shown by *gray arrows*). The POMC precursor is cleaved to form α-melanocyte-stimulating hormone (α-MSH), which binds to melanocortin 1 receptor (MC1R) and stimulates the cAMP–protein kinase A (PKA)–cAMP-responsive element–binding protein (CREB) pathway, leading to microphthalmia-associated transcription factor (MITF) activation. In parallel, KITLG binds to the KIT receptor on the melanocyte, resulting in mitogen-activated protein kinase (MAPK) activity, which also targets MITF. MC1R activation can be blocked by agouti signaling protein (ASIP) or influenced by β-defensin 3 (HBD3) as a partial agonist of the receptor. The MITF and interferon regulatory factor 4 (IRF4) transcription factors lead to the activation of the melanogenic genes, such as *TYR*, and the proteins produced are incorporated into the melanosome. Melanogenesis takes place in the melanosome, which matures into melanin-filled granules as they migrate toward the cell membrane (shown by *black arrows*) and are transported to the keratinocyte cells either singly or in multivesicular bodies. On the left is a representation of the morphology and content of melanosomes by shape and color of European, Asian, and African skin, each in single keratinocytes. The melanosomes form a cap over the nucleus of the keratinocyte cell to serve as photoprotection against UV radiation.

skin pigmentation backgrounds confirmed that a greater range of pheomelanin:eumelanin ratios may appear in the skin (130, 154).

HUMAN ALBINISM

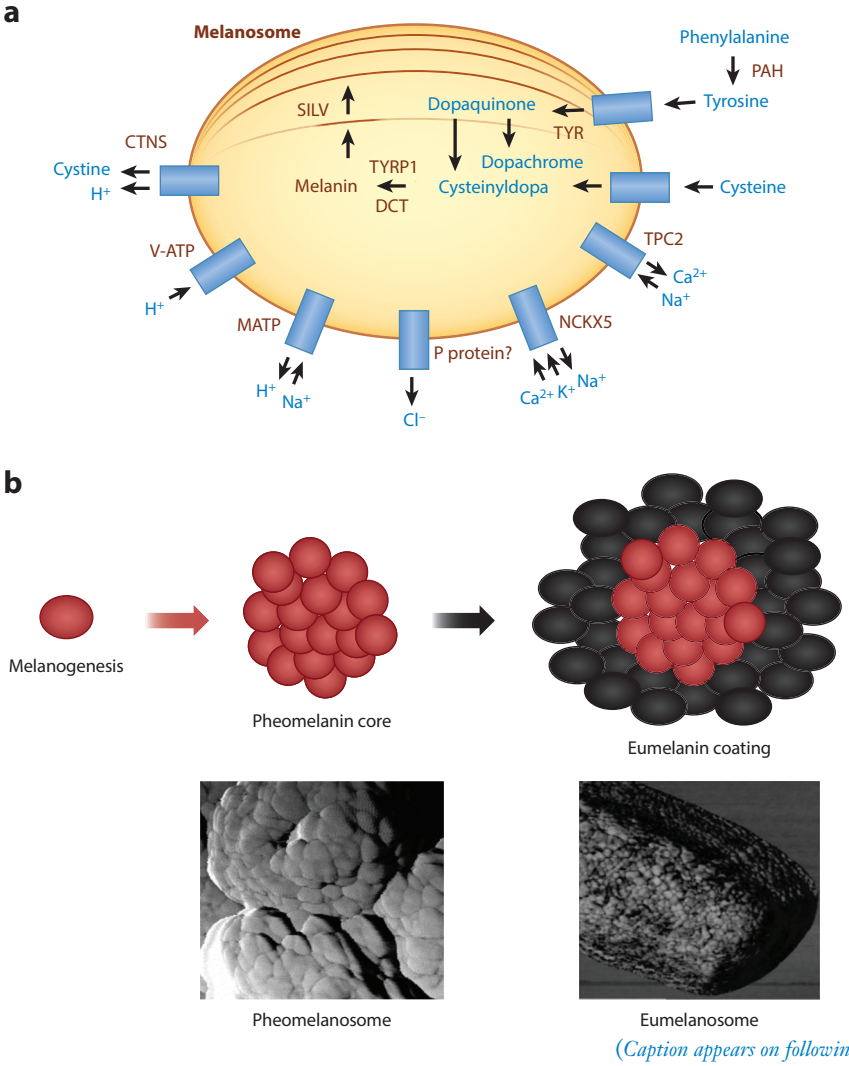
Much of our early knowledge about skin color has come from the study of albinism in mammalian species, which causes a reduction or absence of melanin in the skin, hair, and eyes, as is seen in the dilution of mouse coat colors. This genetic condition is produced by recessive mutations that can occur in 19 currently identified genes linked to hypopigmentation (see below), resulting in defective melanogenesis but preserving the melanocyte cells, unlike piebaldism or vitiligo, where melanocytes are lost. In European populations, oculocutaneous albinism (OCA) appears at a frequency of approximately 1 in 17,000 (103); however, the rates differ significantly around the globe and can approach 1 in 100 in some isolated ethnic populations. One issue is that OCA may be underdiagnosed in light-skinned Europeans compared with family groups of darker pigmentation. Considering this, a higher incidence of 1 in 10,000 of those with European ancestry would give a carrier frequency of 1% for a deleterious albinism gene. This widespread level of pigmentation gene mutation should be considered in obtaining a full understanding of human skin and hair diversity, with the appearance of outlier phenotypes to be expected.

Albinism has been classically divided into three groups: OCA [comprising OCA types 1–7 (OCA1–7), defined by which gene is mutated], ocular albinism [comprising ocular albinism type 1 and foveal hypoplasia, optic nerve decussation defects, and anterior segment dysgenesis (FHONDA)], and syndromic albinism [comprising Chediak–Higashi syndrome type 1 and Hermansky–Pudlak syndrome types 1–9]. These disorders exhibit clinical heterogeneity in the degree of hypopigmentation, ranging from complete absence to mild or even normal cutaneous and follicular coloration. Most forms of OCA, with the exception of OCA3, are characterized by some degree of ocular defects, including nystagmus, foveal hypoplasia, and photophobia. This wide phenotypic variability of patients means that phenotype–genotype correlations are

not straightforward, with the exception of a complete loss of all body melanin in the case of OCA1. This has led some to question the definition of albinism as always including pigmentation defects (104). With the overlap of different forms of the disease and increasing use of genomics in medicine, diagnosis will increasingly require comprehensive molecular analysis.

Albinism Gene Function and Normal Variation in Populations

A recent report has described the 19 albinism-related genes using next-generation DNA sequencing and high-resolution comparative genomic hybridization in 990 affected patients from France (but drawn from countries worldwide) (86). This work allowed molecular diagnosis of 72.3% of this large sample group of patients, with intragenic rearrangements representing 10.8% of pathogenic alleles. In this review, we consider only nonsyndromic OCA genes in relation to skin and hair pigmentation defects and normal variation. In these index patients, OCA1 represented



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

(*a*) Regulation of melanogenesis by substrate and ion transport into the melanosome. Blue rectangles represent ion and amino acid transporters. The conversion of phenylalanine to tyrosine by phenylalanine hydroxylase (PAH) occurs in the cytoplasm of melanocytes and maintains the supply of tyrosine that is actively transported into the melanosome to initiate melanogenesis. Melanogenesis then occurs through the oxidation of tyrosine into dopaquinone by tyrosinase (TYR), with the involvement of tyrosinase-related protein 1 (TYRP1) and dopachrome tautomerase (DCT); dopachrome and cysteinyldopa are key intermediates for eumelanin and pheomelanin, respectively. Ion transport is critical for melanogenesis, with TYR activity being pH dependent. The coupling of H^+, Na^+, Ca^{2+}, K^+, and Cl^- transport by the vacuolar ATP (V-ATP) complex, membrane-associated transporter protein (MATP), P protein, K^+-dependent Na+/Ca^{2+} exchanger 5 (NCKX5), and two-pore segment channel 2 (TPC2) transporters occurs in a complex interconnected regulatory network. Cysteine is also actively transported into the melanosome, and cystine acting as a negative regulator is pumped out by cystinosin (CTNS). The silver locus protein homolog (SILV) forms the matrix in which the melanin compound is polymerized. (*b*) The casing model for mixed melanogenesis. It has been proposed that the default pathway initiating melanin production is pheomelanogenesis, which produces pheomelanin in the melanosome until the cysteine is exhausted. Once this has occurred, eumelanogenesis proceeds, and eumelanin is deposited on the initiating pheomelanic granular core. The two lower images show the spherical pheomelanosome (rough surface) produced in red hair compared with the larger, elongated eumelanosome (smooth surface) from black hair, visualized by atomic force microscopy. Images in panel *b* reproduced from Reference 97 with permission.

41.8% of mutations; OCA2, 27.9%; OCA3, 2.1%; OCA4, 10.5%; OCA6, 3.1%; and OCA7, 0.4% (86). In 12.1% of patients, only one heterozygous mutation was found, and 15.6% failed to resolve any molecular lesion; as such, other albinism genes remain to be identified (104). Notably, OCA5 has been reported in only one Pakistani family (76), with the affected locus mapped to chromosome 4q24, but the gene responsible remains to be identified and screened in this collection. While pathogenic variations in these genes appear in the case of OCA subtypes, there are likely to be polymorphic alleles segregating in human populations that contribute to pigmentary variation observed within the general population (94) and among major population groups (124).

OCA1, OCA3, and the Tyrosinase-Related Protein Family

TYR was one of the first human pigmentation genes identified with mutations responsible for OCA1, which represents the most frequent form of this recessive disease. The TYR enzyme catalyzes the first step of melanin pigment production, the oxidation of its tyrosine or 3,4-dihydroxyphenylalanine (dopa) substrate to form the intermediate dopaquinone (**Figures 1*a*** and **2*a***). Tyrosinase-related protein 1 (TYRP1) is also thought to encode a protein involved in the melanogenic pathway; although its exact enzymatic role in human melanogenesis is unclear (37), complete-loss-of-function mutations of *TYRP1* lead to OCA3. Black South African patients with such deleterious *TYRP1* mutations, for example, have red-bronze skin color and ginger-red hair, a condition termed rufous albinism, but the mutations have less effect on visual acuity than mutations that cause other types of OCA (29, 128). A third member of this family, TYRP2 [now known as dopachrome tautomerase (DCT)], is involved in the isomerization of the red/yellow intermediate dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). This precursor is oxidized into the eumelanin pigment along with 5,6-dihydroxyindole (DHI). However, *DCT* also has a role in protecting melanocytic cells against oxidative stress (6), and its developmental expression pattern is used as one of the earliest markers of melanocyte formation (106). No phenotype has yet been reported in humans for homozygous mutation of the *DCT* locus, but in the mouse, it is recognized as the *slaty* coat color locus, mutation of which results in a dilution of pigmentation.

A boost in understanding the molecular function of these three proteins has come from the recent determination of the crystal structure of the human TYRP1 protein (81), which was then

used to model the common structure of all three TYRP family members (82). The proteins range from 519 to 537 residues in length, with high conservation (24, 143), and have four conserved regions: an N-terminal signal peptide, an intramelanosomal domain, a transmembrane α-helix, and a small C-terminal domain. The active site of each protein comprises four helices that create the binuclear metal-binding sites coordinated by six histidine residues. The TYR active site has two copper ions, and TYRP1 and DCT have two redox-inactive zinc ions. The three-dimensional model of the TYRP family provides a useful tool for the mapping and structural analysis of OCA-related mutations.

Two common polymorphisms in *TYR*, rs1042602*C/A S192Y in exon 1 and rs1126809*G/A R402Q in exon 4, appear at a high frequency in Europeans (**Table 1**) and are largely absent in African populations (71, 137). The 192Y allele is associated with light skin, eye color, absence of freckles, and an increased risk of squamous cell carcinoma, whereas the 402Q change increases the risk of basal cell carcinoma (20) and is frequently associated with albinism patients (29). Protein expression studies have shown that the 402Q variant encodes a thermolabile enzyme (117), and primary melanocyte cultures show that it is retained in the endoplasmic reticulum, hypoglycosylated, and preferentially degraded (69). In the structural model of the TYR protein, the R402 residue is surface exposed and forms a hydrogen bond with Q399 (82). The R402Q change may weaken this interaction, causing TYR to (partially) unfold at higher temperatures but not at lower temperatures.

Recent studies have shown that *TYR* genotype is likely to be a significant modifier of other pigmentation gene polymorphisms in human skin, hair, and eye color, and though not apparent by body site, there is potential for thermal changes in pigmentation of the skin and hair (69). The two polymorphisms were present on four *TYR* haplotypes: the wild type (192S-402R), two single variants (192Y-402R and 192S-402Q), and a double-variant (192Y-402Q) that occurs at a low frequency (1.9%). The double-variant 192Y-402Q haplotype is likely to be deleterious and may explain the association of the 402Q single-nucleotide polymorphism (SNP) with albinism and hypopigmentation in the general population (69, 113). Notably, the R402Q change as a compound heterozygote was found in 46% of OCA1 cases reported by Lasseaux et al. (86).

TYRP1 stabilizes the TYR protein and forms heterodimeric complexes within the melanosome (79). Studies have found that 95% of OCA3-related mutations result in the generation of premature stop codons or frameshifts that produce truncated proteins, red-toned hair, and reddish-brown pigmented skin in black South African patients and optical features that are not as severe as those in other forms of OCA (72, 128). *TYRP1* plays a role in the eumelanin pathway, which occurs at high levels in individuals of African and Oceanic descent. A single SNP, rs1408799, showed genome-wide significant association with blue versus nonblue eyes in Icelandic and Dutch samples. This SNP also had a suggestive association with blond versus brown hair (94, 145). A C93R amino acid change at a highly conserved residue in *TYRP1* is a source of blond hair in Solomon Islanders (77) and northern-island Melanesians (114). The novel mutation rs387907171*C/T occurred at a frequency of 26% in the Solomon Islands but was absent outside Oceania. It is notable that at the same position there is a C93H polymorphism present at 1.1% frequency in the European population (91), but an association with a pigmentation phenotype is yet to be examined.

The melanogenic enzyme DCT is involved in the formation of the photoprotective skin pigment eumelanin through the isomerization of the red/yellow intermediate dopachrome to DHICA. This precursor is oxidized into the eumelanin pigment along with DHI, providing protection against the damaging effects of UV radiation on the skin. However, *DCT* also plays a role in the melanogenic cell response to apoptotic stimuli and oxidative stress (6) and appears as one of the earliest markers of melanocyte formation during development. *DCT* has shown significant differences in genetic variation between Europeans, Africans, and Asians, with local positive selection

Table 1 Human albinism genes and normal phenotypic variation

Albinism	Gene	Protein	SNP[a]	Codon/position/ haplotype	Amino acid/isoform	Allele frequency (%)[b]	Skin[c]	Hair[c]	Eye[c]	Reference for cultured primary melanocytes
OCA1	*TYR*	Melanogenic enzyme	rs1042602*C/A	Coding	Ser192Tyr	63.60/36.40	+	+	+	69
			rs1126809*G/A		Arg402Gln	72.72/27.28				
			rs1042602*C/A-rs1126809*G/A	C-G	192Ser-402Arg	35.0[d]				
				C-A	192Ser-402Gln	32.0[d]				
				A-G	192Tyr-402Arg	30.0[d]				
				A-A	192Tyr-402Gln	1.9[d]	+++	+++	+++	
OCA2	*OCA2/HERC2*	Membrane transporter (P protein)	rs12913832*T/C	5′ distal/*HERC2* intron 86/BEH2	NA	19.60/80.40	+	+	+++	31, 152
			rs7495174*T/C	OCA2 intron 1/BEH1	NA	94.70/5.30	+	+	++	
			rs4778241*G/T			84.03/15.97				
			rs4778138*T/C			87.69/12.31				
			rs1448484*A/G	OCA2 intron 2	NA	99.73/0.27				
			rs1800401*G/A	Coding	Arg305Trp	94.95/5.05	+		+++	
			rs1800407*C/T		Arg419Gln	93.50/6.50				
			rs121918166*G/A		Val443Ile	99.49/0.51				
			rs74653330*C/T		Ala481Thr	99.72/0.28 95.27/4.73[e]	+++			
			rs1800414*T/C		His615Arg	99.98/0.02 40.05/59.95[f]	+++			

(*Continued*)

Table 1 (*Continued*)

Albinism	Gene	Protein	SNP[a]	Codon/position/ haplotype	Amino acid/isoform	Allele frequency (%)[b]	Skin[c]	Hair[c]	Eye[c]	Reference for cultured primary melanocytes
OCA3	*TYRP1*	Melanogenic enzyme	rs1408799*T/C	Noncoding	NA	32.64/67.36	+	+	+	92
			rs387907171*C/T	Coding	Arg93Cys	74.00/26.00[g]		+++		
			rs61752937*G/A		Arg93His	98.89/1.11				
OCA4	*SLC45A2*	Membrane transporter (MATP)	rs26722*G/A	Coding	Glu272Lys	98.02/1.98 60.29/39.71[f]	+			19, 31
			rs16891982*G/C		Leu374Phe	4.36/95.64	+++	+	+	
OCA5	Unidentified	Autosomal recessive	Ch4q24	NA	NA	NA				NR
OCA6	*SLC24A5*	Membrane transporter (NCKX5)	rs1426654*G/A	Coding	Ala111Thr	0.36/99.64	+++	+	+	31
OCA7	*LRMDA*	Leucine-rich melanocyte differentiation–associated protein	rs11001536*A/G	Intronic	NA	97.72/2.28 68.90/31.10[f]	+			NR
			rs35349706*C/T	Coding	Ser153Phe	98.70/1.30 90.12/9.88[h]				
			rs75852090*G/A		Gly176Glu	99.98/0.02				
			rs7923619*G/A	Intronic	NA	90.16/9.84[h]				

Abbreviations: BEH, blue-eye associated haplotype; OCA, oculocutaneous albinism; NA, not applicable; NR, no reference; SNP, single-nucleotide polymorphism.

[a]Major allele/minor allele frequencies in European non-Finnish populations. TYR lists the haplotype frequency only.

[b]Major allele/minor allele frequencies in European non-Finnish populations, with data from Lek et al. (91) and the Exome Aggregation Consortium (**http://exac.broadinstitute.org**), except where indicated by a separate footnote.

[c]Semiquantitative assessment of phenotypic effect: +, weak; ++, medium; +++, strong; blank, unknown.

[d]Data from Jagirdar et al. (69).

[e]Data from the Genome Aggregation Database (gnomAD; **http://gnomad.broadinstitute.org**) for European Finnish populations.

[f]Data from gnomAD for East Asian populations.

[g]Data from Kenny et al. (77) for the Solomon Islands population.

[h]Data from gnomAD for African populations.

in Asians but scarce signals in Africans (8, 85). However, the overall picture reflects a complex pattern of selection, which might include overdominance for *DCT* in Europeans (8). Analysis of data from the 1000 Genomes Project (1) confirms the existence of several noncoding-region polymorphisms with higher-than-expected heterozygosity in the extended *DCT* region in Europeans (8). The SNPs rs1407995*T/C and rs2031526*A/G are *DCT* intronic polymorphisms that each show frequencies of 13.64% in East Asian populations, indicative of linkage. The effects of these SNPs on pigmentation phenotypes are uncertain, but natural variations in *DCT* expression lead us to suspect that the *DCT* locus exhibits genetic interactions with other pigmentation loci (49).

OCA2: P Protein

OCA2 encodes the P protein, which has many functions in pigmentation. It assists in the trafficking and processing of TYR (119) and is potentially involved in tyrosine transport (107), the regulation of melanosomal pH, and glutathione metabolism (134). It also has a role in anion transport by increasing chloride conductance from the melanosome (15). The gene spans more than 345 kb and is divided into 24 exons, 23 of which span the 836-amino-acid coding region containing 12 transmembrane domains, with exon 1 representing a noncoding 5′ untranslated region (90). OCA2 is the second-most-common form of albinism in Europeans and the most common in African populations, who have a frequent 2.7-kb intragenic deletion (Del Ex7) (47, 133). Nonsynonymous coding-region polymorphisms R305W (rs1800401), R419Q (rs1800407), A481T (rs74653330), and V443I (rs121918166) are associated with eye color (10, 44, 140) and skin color (10, 31, 44, 140). The A481T and H615R (rs1800414) alleles are more common in Asian populations and associated with skin lightening (3, 48, 115, 159). The rs1448484*A/G SNP in the second intron of the gene is highly predictive for skin color (99); the A allele is almost monomorphic in Europeans, with the G allele at a frequency of 70.8% in Africans.

A major finding is that regulation of *OCA2* transcription underlies the mechanism determining blue or brown eye color. Initially, genetic association studies discovered a three-SNP haplotype within intron 1 (**Table 1**) of the *OCA2* gene that was highly associated with eye color (44). A haplotype based on rs7495174*T/C, rs4778241*G/T, and rs4778138*T/C [termed blue-eye associated haplotype 1 (BEH1)] was a recessive modifier associated with lighter pigmentary phenotypes (in this study, TGT homozygotes represented 90.5% of subjects with blue/green eye color, 40.2% of those with light brown hair, and 92.6% of those with fair/pale and medium skin). Refined mapping studies of this region found that a key determinant SNP, rs12913832*T/C, located within intron 86 of the adjacent HECT and RLD domain–containing E3 ubiquitin protein ligase 2 (*HERC2*) gene, 21 kb upstream from the *OCA2* initiation site, showed the strongest association with eye color. A haplotype based on the rs12913832*C allele (termed BEH2) is highly associated with blue eye color, and the rs12913832*T allele is associated with brown eye color. Haplotype analysis combining the original three SNPs with rs12913832 showed that the latter split the original haplotypes more precisely into eye color groups. Most critically, the C-TGT haplotype (BEH1–BEH2) had a frequency of 91% in blue-eyed individuals and 37% in brown-eyed individuals, whereas the T-TGT haplotype was present in 0.3% of blue-eyed individuals and 15% of brown-eyed individuals in a study from southeast Queensland (136, 140). A later population-based study of 3,432 individuals from 72 populations genotyped SNPs in the *OCA2–HERC2* region, including those previously associated with eye and skin pigmentation (39). BEH1 and BEH2 were found at high frequencies in Europe, with the C-TGT haplotype essentially restricted to Europe and surrounding regions. In this collection, the derived allele of rs1800414*C 615R was found at high frequency only in populations from East Asia (62–76.1%), Southeast Asia (0–54.3%), and western China (15.5–37.5%), where it is associated with lighter skin color. Notably, genotyping ancient

DNA from skeletons found in several archaeological sites has revealed that some pre-European populations had dark skin and hair pigmentation, but with the BEH2 rs12913832*C SNP that leads to blue eye color, a combination not common in today's European population. It seems that this pigmentation trait evolved before that of skin lightening in Mesolithic hunter–gatherers (7, 118).

The suggestion that the BEH2 haplotype influenced *OCA2* regulation as a distal enhancer element (142) was confirmed in transcript analysis using human primary melanocyte cultures, where a strong correlation between rs12913832 alleles and *OCA2* expression levels was demonstrated (31). Extending this observation, Visser et al. (151) reported binding of the transcription factors helicase-like transcription factor (HLTF), lymphoid enhancer-binding factor 1 (LEF1), and microphthalmia-associated transcription factor (MITF) to the BEH2 region in testing primary melanocyte cultures of homozygous rs12913832*C/C and T/T genotypes. Moreover, an increase in the levels of enhancer-associated chromatin marks in the BEH2 region positively correlated with rs12913832*T/T, OCA2 expression, and the level of melanocyte pigmentation. Using chromosome conformation capture, Visser et al. (151, 152) demonstrated a long-range chromatin loop between rs12913832*T/T and the immediate OCA2 promoter, leading to elevated OCA2 expression. By contrast, in the lightly pigmented melanocytes carrying the rs12913832*C/C genotype, chromatin-loop formation, transcription factor recruitment, and OCA2 expression were all reduced.

OCA4, OCA6, and the Solute Carrier Protein Family

Mapping and molecular cloning of the gene responsible for the mouse *underwhite* coat color allowed identification of the human homolog of membrane-associated transporter protein (MATP), a 530-amino-acid protein with 12 putative transmembrane domains (110). The gene *SLC45A2*, which encodes MATP, is a member of the solute carrier (*SLC*) family, which shares homology with sucrose/proton symporters. A recessive mutation in a splice acceptor sequence of the second exon of *SLC45A2* in a hypopigmented patient allowed classification of the OCA4 type of albinism (110), which has now been expanded as many other patients and recessive mutations are characterized (86). Loss of *SLC45A2* function causes misrouting of TYR, similar to the cellular phenotype of OCA2 (33). The *SLC45A2* gene is expressed at high levels in melanocytes and melanomas, with the MATP protein located in melanosomes (19); however, its role as a melanosomal transporter remains unclear (17). The initial report of an rs16891982*G/C polymorphism within the *SLC45A2* gene (110), which results in a missense amino acid change, L374F, found that it has a significant effect on skin and hair color (99, 116, 132). In African and Asian populations, the ancestral 374L allele is near fixation, and in Europeans it is strongly associated with olive skin and dark hair (31). By contrast, the 374F variant is predominant (at a frequency greater than 95%) in light-skinned European populations (135). Another coding polymorphism found in *SLC45A2*, rs26722*G/A E272K, is also associated with skin, eye, and hair pigmentation (56, 131) but is in strong disequilibrium with rs16891982*G/374L in European populations (96).

A polymorphism in another human *SLC* family member, *SLC24A5*, was discovered through the identification of the zebrafish golden mutant pigmentation locus (84). This gene encodes the K^+-dependent Na^+/Ca^{2+} exchanger 5 (NCKX5) protein. The SNP, rs1426654*G/A, which results in an NCKX5 coding change, T111A, has the largest effect on decrease in skin pigmentation in Europeans relative to other population groups. The allele has reached near fixation in the European population, with a frequency approaching 99%; as such, it cannot explain to any significant degree the normal range of intra-European differences in skin color. The relative importance of this allele in the study of skin color diversity in Indian populations has been questioned

(64). The 111A allele frequency increased in ancestral Europeans in the 3,000 years between the Mesolithic and the Bronze Age, while the 111T ancestral allele is again close to fixation in dark-skinned African populations (7, 99, 136). *SLC24A5* gene mutations are associated with a new form of OCA, OCA6 (86, 157). Intracellular Ca^{2+} elicits changes in cellular melanin content, and the Na^{+}/Ca^{2+} exchanger activity of SLC24A5 may provide a link between cytosolic and melanosomal Ca^{2+} signaling by regulating Ca^{2+} transport from the cytosol to the melanosome lumen (55). Ca^{2+} within the melanosome might also have an essential role in activating the proteolytic cleavage of the silver locus protein homolog (SILV) protein, which polymerizes to form the melanosomal matrix (84).

A third member of the *SLC* family, *SLC24A4*, encoding NCKX4, was first reported as a modifier of eye color (144) and is associated with skin and hair color variation in northern Europeans (61). Other members of the *SLC* family may also contribute to melanogenesis (27). SNPs have been reported within the human *SLC7A11* gene, which encodes a cystine/glutamate exchanger and is important for pheomelanin but not eumelanin production in *subtle gray* mutant mice (30).

OCA7: LRMDA

Mutation of the *LRMDA* gene, encoding leucine-rich melanocyte differentiation–associated protein, was identified in families from Denmark and associated with another rare form of OCA, OCA7 (57). *LRMDA* encodes a 198-amino-acid protein containing three leucine-rich repeats and one leucine-rich-repeat C-terminal domain. This family of proteins includes members with a variety of functions, including cell adhesion, signaling, extracellular-matrix assembly, neuronal development, and RNA processing. Knockdown experiments of the orthologous gene in zebrafish demonstrated a reduction in pigment cells and in the amount of pigment per cell, supporting an important role in melanocyte differentiation. The additional patients identified by Lasseaux et al. (86) were consistent with the phenotype of these earlier probands, with characteristic nystagmus, foveal hypoplasia, and iris transillumination. The contribution of *LRMDA* to normal variation in pigmentation has been recognized in a GWAS for eyebrow color, with this trait showing a high degree of variation in Europeans (120). In this study of 6,513 individuals rated for eyebrow color as red, blond, brown, and black, the most highly associated SNP was rs11001536*A/G, with the G allele associated with light eyebrow color.

HUMAN PIGMENTATION GENES: IDENTIFICATION BY COMPARATIVE GENOMICS AND BIOCHEMICAL METHODS

The MC1R/POMC/ASIP/HBD3 Axis

The switch from pheomelanin to eumelanin synthesis by melanocytes in the determination of human skin and hair pigmentation characteristics (66) was first established through the genetic characterization of the *extension* locus of mice (127), which encodes the receptor that binds α-melanocyte-stimulating hormone (α-MSH). The murine melanocortin 1 receptor (Mc1r) is a membrane-bound G protein–coupled receptor (GPCR) that is expressed on the cell surface of melanocytes and is activated by α-MSH to increase cAMP levels within the cell but is blocked by another mouse genetic locus, *Agouti* (87, 88) (**Figure 1*b***). The proopiomelanocortin (POMC) protein is the precursor for both α-MSH and adrenocorticotropic hormone (ACTH), which binds human MC1R with the same affinity and cAMP activation potential (2, 146) and is expressed in the skin and hair follicles. In Addison's disease, corticotropins are continually released from the hypothalamo-pituitary axis, which systemically leads to hyperpigmentation of stimulated melanocytes; loss of corticotropins by mutation of the human *POMC* gene leads to metabolic

deficiencies and red hair (80). A recent study showed that other distinct, noncanonical, cAMP-dependent mechanisms controlling pigmentation operate by regulating melanosomal pH (162).

MC1R is commonly known as the gene for the red hair color phenotype. Population-based studies, predominantly of individuals with European ancestry, have reported that the *MC1R* gene is highly polymorphic, with more than 200 variants identified (52, 149). There are nine common allelic variants (14), but others have been recently recognized (105) (**Table 2**). *MC1R* coding variants known as *R* alleles have a strong association with red hair, fair skin, and freckling as well as increased melanoma and nonmelanoma skin cancer risk. The most common *R* variant alleles are D84E, R142H, R151C, R160W, and D294H (13, 52). *MC1R* alleles that are less penetrant for red hair color are known as *r* alleles, with the consensus wild-type allele having high basal activity (108); the common *r* variants are V60L, V92M, I155T, and R163Q. The *MC1R* genotype has effects that extend beyond pigmentation and involve activation of the DNA damage response and repair (147); tolerance to pain (102); resistance to anesthesia (93); and, potentially, wizardry (126). Notably, there may have been introgression of the V92M allele from Neanderthals into modern human populations outside Africa (36, 38). As a recessive trait, the red hair color phenotype was classically expected to require two *R* alleles in the homozygous or compound heterozygous state; individuals with an *R/R* genotype do have the highest penetrance for red hair, at approximately 70%, but approximately 30% of those with red hair have an *R/r* genotype (41). This could be due to genetic interactions with other pigmentation loci segregating in the population, most notably the gene encoding agouti signaling protein (*ASIP*) (45, 105). Large differences in the distribution of *MC1R* variants across European populations have been described, with the highest frequency of *R* alleles occurring in northern populations (53).

The physiological antagonist for MC1R is ASIP, which has been implicated in pigmentation phenotype variation and melanoma risk through GWASs (96, 98). The *ASIP* gene product is a 131-amino-acid protein that facilitates pheomelanin production (87, 88). The association of melanoma with the *ASIP* region closely parallels that for red hair color and seems to be due largely to a single long (approximately 1.8 Mb) haplotype containing 22 known genes, including *ASIP* (45, 96, 145). The *ASIP* haplotype is tagged by markers rs1015362*G and rs4911414*T, located more than 100 kb upstream of the *ASIP* gene, but is strongly associated with melanoma (58) and pigmentation. Similar to the red hair color variants, the *ASIP* haplotype is associated with freckling, skin sensitivity to sun, and red and blond hair (45, 105). Studies have shown that the rs4911442*C *ASIP* allele is equivalent to an *r MC1R* allele in the penetrance of red hair color, suggesting a gene–gene interaction between *MC1R* and the *ASIP* locus that affects hair color (45, 105).

A third ligand for MC1R is the β-defensin 3 (HBD3) protein secreted by keratinocytes. A mutation in the canine homolog gene, *CBD103*, is associated with black coat color in dogs and the gray wolf (23, 129). Experiments using cultured human melanocytes showed that HBD3 had no effect on cAMP levels but blocked the ability of α-MSH to stimulate cAMP accumulation and TYR expression, as such acting as an MC1R antagonist (148). It may have a potent ability to stimulate mitogen-activated protein kinase (MAPK) (14). A comprehensive structure–function analysis of HBD3–MC1R showed that this ligand interacts in a way that is fundamentally different from α-MSH or ASIP (112). Additional studies are needed to elucidate which signaling pathway HBD3 activates via *MC1R* and the role of UV induction of the HBD3 protein in the skin-tanning response and photoprotection (70).

Genetic variation in the *MC1R* pathway is a key element in directing human skin and hair color, and there are a multitude of interactions with other pigmentation genes in the determination of phenotype. Proportionate lightening occurs in individuals carrying recessive blue eye *OCA2* and red hair *R* alleles, indicating an additive action of *MC1R* and *OCA2* loci on constitutive skin color (41) and the phenotype of OCA2 albinism (78). The modifying effect of *OCA2* on *MC1R* is also

Table 2 Human pigmentation genes with phenotype associations

Gene	Protein	SNP[a]	Codon/position/ haplotype	Amino acid/isoform	Allele frequency (%)[b]	Skin[c]	Hair[c]	Eye[c]	Cultured primary melanocytes
ASIP	MC1R antagonist	rs4911442*T/C	Extended haplotype on chromosome 20	NA	89.12/10.88	+	+	+	70, 148
		rs6059655*G/A	eQTL in *RALY*	NA	89.41/10.59[d]				
BNC2	Basonuclin 2	rs2153271*T/C	Intron 1	NA	55.37/44.63	+			153
		rs10765819*G/A			50.1/49.9				
		rs12350739*A/G	5′ distal	NA	55.22/44.78				
		rs62543565*A/C			59.26/40.74				
DCT	Melanogenic enzyme	rs1407995*C/T	Intron 6	NA	81.83/18.17 24.36/75.64[e]		+		60
		rs2031526*G/A			81.90/18.10 24.33/75.67[e]				
IRF4	Interferon regulatory factor 4	rs12203592*C/T	Intron 4	NA	85.61/14.39	++	++	++	28
KIT (piebaldism)	Growth factor receptor tyrosine kinase	rs115585711*G/A	Coding	Ala178Thr	98.79/1.21[f]	++	++		156
		rs3822214*A/C		Met541Leu	90.31/9.69				
KITLG (familial progressive syndromes)	Growth factor (SCF)	rs12821256*T/C	5′ distal	NA	88.93/11.07	++	++		75
		rs642742*C/T			82.75/17.25 21.31/78.69[f]				
MC1R	G protein–coupled receptor	rs3212379*C/T	5′ distal	NA	99.9/0.1[d]	++	+++		14
		rs1805005*G/T	Coding, nonsynonymous Wild type, *r*, or *R*	Val60Leu (*r*)	12.79				
		rs34474212*T/C		Ser83Pro (*R*)	0.04				
		rs1805006*C/A		Asp84Glu (*R*)	0.88				
		rs2228479*G/A		Val92Met (*r*)	7.86				
		rs34158934*C/T		Thr95Met (*R*)	0.06				
		rs11547464*G/A		Arg142His (*R*)	0.73				
		rs1805007*C/T		Arg151Cys (*R*)	6.98				
		rs201326893*C/A		Tyr152Ter (*R*)	0.05				
		rs1110400*T/C		Ile155Thr (*r*)	0.84				
		rs1805008*C/T		Arg160Trp (*R*)	8.07				
		rs885479*G/A		Arg163Gln (*r*)	5.30				
		rs555179612*T/TC		Ile180Hisfs (*R*)	0.10				
		rs200000734*C/T		Arg213Trp (*R*)	0.05				
		rs1805009*G/C		Asp294His (*R*)	1.38				
		rs368507952*G/A		Arg306His (*R*)	0.01				
		NA		Wild type	~50				

(*Continued*)

Table 2 (*Continued*)

Gene	Protein	SNP[a]	Codon/position/ haplotype	Amino acid/isoform	Allele frequency (%)[b]	Skin[c]	Hair[c]	Eye[c]	Cultured primary melanocytes
MITF	Microphthalmia-associated transcription factor	rs149617956*G/A	Coding	Glu318Lys	99.76/0.24	+	+	+	21
		rs9823839*T/C	Intronic	NA	54.72/45.28		+		
POMC	Proopiomelanocortin	rs76645364*A/G[d]	5′ distal	NA	81.64/18.36[d]		+		70, 148
SLC24A4	Membrane transporter (NCKX4)	rs12896399*G/T	5′ distal	NA	55.77/44.23			+	27
		rs8014907*A/T	Intronic	NA	81.36/18.64				
TPCN2	TPC2	rs72930659*C/T	5′ distal	NA	85.88/14.12		++		9
		rs72928978*G/A	Coding	Val219Ile	87.02/12.98		+		
		rs3750965*A/G		Lys376Arg	69.12/30.88				
		rs35264875*A/T		Met484Leu	81.48/18.52				
		rs3829241*G/A		Gly734Glu	61.37/38.63	+			
		rs4930263*G/A	Intronic	NA	99.8/0.2	+			
		rs2376558*C/T			99.1/0.9	++	++		
		rs10896418*G/T			99.95/0.05				

Abbreviations: eQTL, expression quantitative trait locus; NA, not applicable; SNP, single-nucleotide polymorphism.

[a]Major allele/minor allele frequencies in European non-Finnish populations. For *MC1R*, only the minor variant allele frequency is given.

[b]Major allele/minor allele frequencies in European non-Finnish populations, with data from Lek et al. (91) and the Exome Aggregation Consortium (**http://exac.broadinstitute.org**), except where indicated by a separate footnote.

[c]Semiquantitative assessment of phenotypic effect: +, weak; ++, medium; +++, strong; blank, unknown.

[d]Data from Morgan et al. (105).

[e]Data from the Genome Aggregation Database (gnomAD; **http://gnomad.broadinstitute.org**) for East Asian populations.

[f]Data from gnomAD for African populations.

seen in freckling score; blue eye color increases freckling, and the *R* alleles further increase the freckling score in an additive fashion (11, 41, 139).

MITF

Upon ligand binding to MC1R, expressed on the cell surface of melanocytes, the signaling pathway begins with Gαs activation of adenylate cyclase, leading to intracellular cAMP formation. Protein kinase A (PKA) is stimulated by cAMP, which is followed by phosphorylation of members of the cAMP-responsive element–binding protein (CREB) family of transcription factors. CREB transcriptionally activates a variety of downstream targets, including *MITF* gene expression. *MITF* is a master regulator of melanocyte growth and differentiation (111) that controls many aspects of the pigmentation response, including TYR activity and increased protein levels of TYRP1 and DCT, leading to the biosynthesis of eumelanin pigments.

The MITF protein is itself modulated by a multitude of chemical modifications, including phosphorylation by MAPK induced through c-KIT activation (158). SUMOylation is another reversible posttranslational modification that occurs on the K316 residue of MITF that represses the activity of DNA binding to its target sites (89). A germline missense rs149617956*G/A E318K allele of the *MITF* gene, encoding a hypoSUMOylated variant protein, has been described as a medium-penetrance melanoma gene occurring at a frequency of approximately 0.24% in European populations (18, 160). One study reported that *MITF* E318K carriers were two to five times more likely to develop melanoma and had a phenotype consisting of fair skin, freckling, and high total nevus count (141). The functional effects and molecular mechanisms of action of the MITF E318K protein have been studied both in transgenic mice and in melanocytes derived from heterozygote donors (21). Mutated mice were slightly hypopigmented, and analysis of the cultured melanocytes directly demonstrated a decrease in the cellular level of MITF SUMOylation and a cell senescence delay that may contribute to the risk of melanoma development.

IRF4

A SNP within intron 4 of the interferon regulatory factor 4 (*IRF4*) gene, rs12203592*C/T, has been independently associated with pigmentation and age-specific effects on nevus count in European-derived populations (42). *IRF4* is one of only two genes (together with *TYR*) known to affect skin, eye, hair, nevi, and freckling (61) and is one of the few genes linked to hair graying (4).

IRF4 is a member of the interferon regulatory factor family of transcription factors, which play an important role in immune system development and function. The rs12203592*T polymorphism is strongly associated with darker hair color, lighter eye color, and a reduced skin-tanning response to sunlight (61, 109). The polymorphism lies within an enhancer element that drives *IRF4* transcription in melanocytes (28, 123). Transcription factor AP-2α (TFAP2A) and MITF directly regulate *IRF4* expression, and *TYR* expression depends on the IRF4 protein (28, 123). Asian and African populations are fixed for the rs12203592*C allele, with only European populations possessing rs12203592*T, at an allele frequency of approximately 14.4%. The rs12203592*T SNP is positively associated with melanoma and solar elastosis and statistically significantly associated with having 10 or fewer back nevi, dark hair color, light eye color, and decreased ability to tan (54). European populations with the *IRF4* rs12203592*C/C genotype have a predominantly early onset of melanoma distribution, peaking at approximately age 45 years, while patients with the rs12203592*T/T genotype have a predominantly late-onset distribution, peaking at approximately age 75 years (54). A recent study found that *IRF4* rs12203592 interacts with the *OCA2* BEH2 genotype to influence both eye color and the number of iris freckles (83).

Analysis of primary melanocytic cells has revealed a link between the *IRF4* genotype and growth, pigmentation, and survival in response to UV radiation. Homozygous rs12203592*T/T melanocyte strains do not respond to the interferon γ (IFN-γ) cytokine, which then potentially influences melanomagenesis and immune evasion (28).

KIT and KITLG

Piebaldism is an autosomal dominant disorder that is caused by altered proliferation and migration of melanocyte precursor cells and presents as white-spotted patches of the skin or hair. This genetic condition has been associated with mutations in the *c-KIT* gene, encoding a tyrosine kinase receptor (122). Normal variation in this gene, such as rs3822214*A/C M541L at an allele frequency of approximately 9.7% (**Table 2**), occurs but does not have obvious population-specific or phenotypic associations. However, a clinically significant A178T allele is seen in the African population at a frequency of 1.2%.

The gene encoding the ligand for the KIT receptor, *KITLG*, regulates the number of melanocytes during development, melanin distribution in the skin, and onset of familial progressive syndromes of both hyper- and hypopigmentation (122, 144). A transversion (c.107A>G) in exon 2 of *KITLG* is responsible for inherited familial progressive hyperpigmentation, producing a gain-of-function defect in TYR activity and melanin synthesis (122). The first report that polymorphism of *KITLG* could be associated with human skin color was the finding that rs642742*T/C is associated with a higher melanin index in an African American population (101). This was followed by the discovery that rs12821256*T/C SNP, located in a large intergenic region more than 350 kb upstream of the *KITLG* transcription start site, alters the binding site for the LEF transcription factor. This reduces LEF responsiveness and enhancer activity in cultured human keratinocytes (59), affecting the expression of *KITLG* mRNA. The SNP rs12821256*T/C is genetically associated with blond hair in northern European countries, such as Iceland and the Netherlands, at a frequency of 11.1% (51, 59, 61, 94, 137, 144).

TPCN2

In a GWAS of 8,460 Icelandic and Dutch individuals (144), two common coding-region SNPs, rs35264875*A/T M484L and rs3829241*G/A G734E, were strongly associated with hair color in comparing blond versus brown hair. These polymorphisms are within the two-pore segment channel 2 gene (*TPCN2*), encoding the TPC2 protein, which is thought to be a cation selective ion channel (9). Two other SNPs, rs72928978*G/A V219I (common in the European population) and rs3750965*A/G K376R (present in all populations), are also associated with hair color in the southeast Queensland population (43) but have less effect (**Table 2**); other intronic SNPs in this region are also strongly associated with skin color, suggesting regulation of the expression of the *TPCN2* transcript (D.L. Duffy & R.A. Sturm, unpublished results).

The TPC2 protein is expressed in melanocytes, and a study by Ambrosio et al. (9) used immunological methods to localize it to endolysosomal compartments, including the melanosome. Knockout of the gene caused a substantial increase in melanin content in both melanoma cells and primary human melanocytes, with the melanosomal lumen less acidic in the knockout. The data from Ambrosio et al. (9) showed that TPC2 likely regulates melanosome pH and size by mediating Ca^{2+} release from the organelle. The organellar signaling lipid phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$] has been implicated in the modulation of TPC2 function to control melanosomal pH, resulting in acidification and decreased pigmentation (16). Later work examining the function of the coding alleles M484L and G734E found that both polymorphisms lead to

a gain of channel function by independent mechanisms (26). Expression of the 484L form of the TPC2 protein demonstrated that the sensitivity to its endogenous ligand, $PI(3,5)P_2$, is strongly increased, and the mutation also leads to a structural change that affects pore dynamics, while with the 734E allele channel, inactivation by ATP is reduced. The increase in the channel activity of these alleles would lead to acidification of the melanosome and a decrease in melanin production, explaining the shift from brown to blond hair in carriers of these alleles.

BNC2

An rs2153271*T/C polymorphism within the gene encoding the basonuclin 2 protein (*BNC2*), a zinc-finger-motif DNA-binding factor, was first identified in a large genetic association study for common traits, including freckling (51). Later analysis of candidate pigmentation-related genes with digital images of skin color found that the rs10756819*G/A SNP is associated with the quantitation of saturation of the image (68). The expression of the *BNC2* gene using human melanocyte cell lines derived from donors of different skin color has allowed a correlation of genotype with phenotype (153). Using chromatin immunoprecipitation for an active marker of transcription acetylation of histone H3 K27 (H3K27Ac) combined with chromatin immunoprecipitation sequencing (ChIP-seq), Visser et al. (153) mapped a 5′ regulatory element of the *BNC2* gene and identified a possible causative SNP, rs12350739*A/G, that functions as an enhancer region for *BNC2* transcription in human melanocytes. The expression of BNC2 was higher in the dark and moderately pigmented cells, associated with rs12350739*G, when compared with the expression of BNC2 in the light pigmented cells, associated with rs12350739*A. Analysis of epidermal samples showed that the rs12350739*A/A genotype corresponds with a light skin pigmentation phenotype, whereas the rs12350739*G/G genotype, in which the enhancer is more accessible in the chromatin, corresponds with a dark skin pigmentation phenotype. A GWAS conducted on 3,443 Dutch individuals using digital facial photographs associated four genes, *IRF4*, *MC1R*, *ASIP*, and *BNC2*, with pigmented aging spots (solar lentigines and seborrheic keratosis) and found that rs62543565*C/A was the most significant SNP in *BNC2* (67). These variants contributing to facial aging pigment changes are thought to act via pathways independent of basal melanin production. A recent study of the genetic contribution of Neanderthals to phenotypic variation in modern humans has recognized the introgression of two BNC2 haplotypes, one associated with sun sensitivity and the other with darker skin pigmentation (36).

OUTCOMES OF GENOME-WIDE ASSOCIATION STUDIES: IDENTIFICATION OF NEW PIGMENTATION GENES

Studies of African and Latin American Populations

The genetic basis of skin pigmentation has recently been extended to more ethnically diverse populations (34, 100). Crawford et al. (34) assessed cutaneous pigmentation in a population of 2,092 ethnically and genetically diverse Africans living in Ethiopia, Tanzania, and Botswana by quantification for light reflectance and genotyped them for a GWAS. In African genomes, they identified variants significantly associated with skin pigmentation near two previously identified genes (*SLC24A5* and *OCA2/HERC2*) and two new loci [major facilitator superfamily domain containing (*MFSD12*) and a 195-kb region on chromosome 11 encompassing the damage-specific DNA-binding protein 1 (*DDB1*) and transmembrane protein 138 (*TMEM138*) genes]. Even though the study included only 2,092 individuals, the authors were able to account for 28.9% of the genetic variance, with a trait variation attributable to each locus of 12.8%, 3.9%, 4.5%, and 2.2%,

respectively. Interestingly, the genomic architecture consists of variants potentially introduced from non-African populations (*SLC24A5*) as well as variants unique to African descendants. The strongest association was with *SLC24A5*. The rs14266654*A variant was associated with lighter skin color in non-African populations and was introduced into East Africa by gene flow from outside of Africa (95). It is a common variant in African populations, occurring at a high frequency (28–50%) in highly Afro-Asiatic populations (Ethiopia and Tanzania), a moderate frequency (5–11%) in Botswanan San and Bantu-speaking populations, and a low frequency in populations with East African ancestry. A demonstration of the power of including diverse populations in genetic studies, this work also identified a new pigmentation gene, *MFSD12*, and attributed eight potential causative noncoding SNPs to this locus. The derived alleles of the two SNPs with the highest probability, rs56203814*T and rs10424065*T, are associated with darker pigmentation, present at a much higher frequency in African populations, and common in Nilo-Saharan East Africans (**Table 3**). The ancestral alleles at two other loci, rs6510760*G and rs112332856*T, are associated with lighter pigmentation. Genomic analyses of these SNPs indicated that the derived alleles are associated with decreased mRNA of *MFSD12* and increased pigmentation. Analyses of cells in culture are consistent with this, with knockdown leading to increased pigmentation. Interestingly, its localization is more consistent with lysosomal than melanosomal vesicles. Analysis of spontaneous and genetically engineered mouse models demonstrated that disruption of *Mfsd12* results in reduced pheomelanin in the fur of *agouti* mice. The remaining new genomic region is a 195-kb cluster of genes on chromosome 11 that includes *DDB1* and *TMEM138* and may involve UV response and DNA repair. Interestingly, variants associated with dark pigmentation from this study in Africans were also shown to be identical by descent with those of people in South Asian and Australo-Melanesian populations.

A study by Martin et al. (100) also described the complex nature of pigmentation genetics. Examining the genetic contributions to pigment variation in KhoeSan populations indigenous to southern Africa, they focused on a population that has considerably lighter skin than Africans located closer to the equator. They used whole-genome genotyping and targeted resequencing of pigmentation loci in 465 people (278 Khomani San and 187 Nama). As might be expected, they found that skin pigmentation is a heritable trait and that some of the known pigmentation loci explain the pigment lightening, with *SLC24A5* and *OCA2* showing strong selection. However, they found that only a small fraction of the genetic variance was due to these and other known pigment genes, such as *TYRP1* and SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2 (*SMARCA2*)/very-low-density lipoprotein receptor (*VLDLR*), and novel loci, such as sorting nexin 13 (*SNX13*) (**Table 3**). These studies reinforce the complex nature of skin pigmentation, which depends on the population studied and the underlying local selective pressures.

Following on from these African studies, Adhikari et al. (5) conducted another large population-based GWAS for skin, hair, and eye pigmentation qualities in more than 6,000 individuals from Latin America. This cohort had high Native American ancestry drawn from five countries: Brazil, Colombia, Chile, Mexico, and Peru. It is known that Native Americans are closely related to East Asians due to the entry of founding populations into the Americas from eastern Siberia some 15,000 years ago, which was followed by migration throughout the continent. A large proportion of the phenotypic variation in this sample was explained by the established pigmentation gene variants, including *TYR*, *TYRP1*, *OCA2/HERC2*, *SLC45A2*, *SLC24A5*, and *IRF4*. However, a novel region associated with skin color included the intergenic rs11198112*C/T SNP close to the *EXM2* gene, a new candidate also identified in the skin-tanning response (150). The strongest association for skin color was observed within the *MFSD12* gene tagged by the rs2240751*A/G nonsynonymous Y182H variant, which is common only in Native Americans and

Table 3 Newly associated human pigmentation genes

Gene	Protein	SNP[a]	Codon/position/ haplotype	Allele frequency (%)[b]	Skin[c]	Hair[c]	Tanning response[c]	Reference
SLC45A1	Solute carrier	rs80293268*G/C	NA	94.18/5.82		+		63
DSTYK	Protein kinase	rs2369633*T/C	NA	9.78/90.22		+		63
FOSL2	Transcription factor subunit	rs71443018*G/C	NA	94.03/5.97		+		63
LHX2	Homeobox transcription factor	rs58979150*C/T	NA	89.53/10.47		+		63
EDNRB	Transmembrane receptor (endothelin)	rs1279403*C/T	NA	38.79/61.21		+		63
KRT31	Keratin	rs117612447*C/T	Splice donor variant	97.14/2.86		+		63
BCAS1	Unknown	rs73132911*T/C	NA	94.94/5.06		+		63
FGF5	Growth factor	rs7681907*G/A	NA	57.00/43.00		+		63
SOX5	Transcription factor	rs9971729*A/C	NA	42.63/57.37		+		63
TWIST2	Transcription factor	rs11684254*C/G	NA	65.61/34.39		+		63
TSPAN10	Oculospanin	rs6420484 *A/G	Y177C	35.95/64.05		+		150
PDE4B	Phosphodiesterase	rs1308048*T/C	NA	56.76/43.24			+	150
RIPK5	Phosphatidylinositol phosphate kinase	rs12078075*G/A	NA	9.29/90.71			+	150
PA2G4P4	Pseudogene	rs9818780*T/C	NA	54.15/45.85			+	150
PPARGC1B	Peroxisome proliferator-activated receptor gamma coactivator 1-beta	rs251464*G/C	NA	72.86/27.14			+	150
AHR/AGR3	Aryl hydrocarbon receptor/disulfide isomerase	rs117132860*G/A	NA	98.15/1.85			+	150
TRPS1	Transcription repressor	rs2737212*C/T	NA	43.53/56.47			+	150
EMX2	Homeobox transcription factor	rs35563099*C/T	NA	84.34/15.66			+	150
		rs11198112*C/T	NA	84.29/15.71	++			5
ATP11A	ATPase	rs1046793*C/T	NA	44.82/55.18			+	150
KIAA0930	Unknown	rs11703668*A/G	NA	51.00/49.00			+	150
BNC2	Zinc-finger protein	rs10810650*C/T	NA	44.49/55.51			+	150

(*Continued*)

Table 3 (*Continued*)

Gene	Protein	SNP[a]	Codon/position/ haplotype	Allele frequency (%)[b]	Skin[c]	Hair[c]	Tanning response[c]	Reference
MFSD12	Solute transporter (major facilitator superfamily)	rs56203814*C/T	NA	99.15/0.85 84.14/15.86[d]	+			34
		rs10424065*C/T		99.20/0.80 77.82/22.18[d]				
		rs6510760*G/A		92.88/7.12 29.35/70.65[d]				
		rs112332856*T/C		96.65/3.35 36.63/63.37[d]				
		rs2240751*A/G	Y182H	99.01/0.99 73.28/26.72[e]	++			5
		rs77733715*A/G	NA	98.52/1.48 63.81/36.19[e]			++	138
DDB1/TMEM138	DNA damage binding/transmembrane protein	rs7948623*T/A	NA	0.29/99.71 21.47/78.53[d]	+			34
SMARCA2/VLDLR	Transcriptional activator	rs10962731*G/A	NA	99.89/0.11 84.21/15.79[d]	+			100
	Very-low-density lipoprotein receptor	rs872257*G/A		27.26/72.74 61.97/38.03[d]				
SNX13	Sorting nexin	rs2110015*C/T	NA	46.37/53.63 18.22/81.78[e] 38.82/61.18[d]	+			100

Abbreviation: NA, not applicable.

[a] Reference allele/alternate allele frequencies in European non-Finnish populations.

[b] Reference allele/alternate allele frequencies in European non-Finnish populations, with data from Lek et al. (91) and the Exome Aggregation Consortium (**http://exac.broadinstitute.org**).

[c] Semiquantitative assessment of phenotypic effect: +, weak; ++, medium; +++, strong; blank, unknown.

[d] Data from the Genome Aggregation Database (gnomAD; **http://gnomad.broadinstitute.org**) for African populations.

[e] Data from gnomAD for East Asian populations.

East Asians (**Table 3**). Follow-up analyses have determined that the *MFSD12* region has undergone significant evolutionary selection in East Asians, with the frequency of the Y182H polymorphism correlating with the intensity of solar radiation and lightening of skin color.

Together, these three GWAS approaches illustrate that higher-powered studies with increasingly diverse populations can successfully identify additional genetic determinants contributing to pigmentation in human populations.

Cell-Based Assays for Pigmentation Genes

Cell-based functional genomic and computational studies are also adding to our ability to identify the functional variants underlying pigmentation GWAS peaks. Expression quantitative trait locus (eQTL) analysis is one way to link gene expression with variations in regulatory regions. Numerous tissues have been used for eQTL analyses and are publicly available. For example, the Genotype-Tissue Expression (GTEx) project has data generated from more than 40 postmortem human samples, including sun- and non-sun-exposed skin (50). Recently, eQTL analyses were generated from 106 newborn male primary melanocyte cultures to develop a resource of transcriptional variation linked to genomic differences (161). Strikingly, given the small sample size, the project was highly successful, yielding 597,335 *cis*-eQTL SNPs and 4,997 eGenes (genes whose expression levels are associated with one or more eQTLs). Comparison with GTEx data showed that using melanocyte eQTLs was a more powerful way to identify genes involved in pigmentation than using skin samples, most likely because analyzing melanocyte cell cultures devoid of other skin cell types reduces the cellular complexity. This study also identified eGenes that are candidates for melanoma and pigmentation GWAS loci, including *OCA2*, *DCT*, *SLC45A2*, *TYR*, *MC1R*, *IRF4*, cystinosin (*CTNS*), and myosin 5A (*MYO5A*).

The use of these data sets and expansion to other cell types of the skin, such as keratinocytes, will help to further dissect the underlying genetic causes of pigment variation. Integrating the extensive work in animal models will also be useful in identifying and characterizing genes involved in human pigmentation; however, the data are often found at different websites and with different descriptors. Baxter et al. (12) recently compiled a comprehensive, cross-species list of 650 genes involved in pigmentation phenotypes from manually curated genes annotated in the Online Mendelian Inheritance in Man (OMIM), Mouse Genome Informatics (MGI), Zebrafish Information Network (ZFIN), and Gene Ontology (GO) databases. This growing data set will also help to prioritize candidate genes underlying GWAS peaks.

Genes for Eye Color

Duffy (40) recently published an extensive review of the genetics of eye color, which included a discussion of the involvement of the *OCA2* gene described in detail above, so only a brief summary is provided here. Similar to hair and skin color, the range of eye color is controlled by the amount and ratio of eumelanin and pheomelanin production by neural-crest-derived melanocytes in the iris. However, unlike skin and hair, where pigment-containing melanosome granules affect coloration by their transfer to the keratinocytes of hair shafts or epidermis, in the iris melanosomes containing pigment are retained within the melanocytes. Blue and green eyes have a larger ratio of pheomelanin to eumelanin (~14:1), while brown eyes contain similar amounts of each pigment. Eye color is highly heritable, with three-fourths of the genetic variance attributable to a subset of the genes involved in skin and hair pigmentation: *IRF4*, *OCA2*, *SLC24A4*, *SLC24A5*, *SLC45A2*, *TYR*, and *TYRP1*. Unique to eye pigmentation, melanocytes are not regulated by the skin pigmentation genes *KITLG*, *MC1R*, and *ASIP* and do not respond to UV with a tanning response.

Genome-Wide Association Studies for Hair Color and Tanning

Hair follicles are complex organs that have a diverse three-dimensional cell structure and are formed from multiple cell lineages that cycle during hair shaft growth and regression. The bulk of the hair follicle is composed of keratinocytes, with the base consisting of a specialized population of dermal papilla cells that interact with melanocytes embedded in the follicular epithelium and provide pigment to the forming hair shaft. The dermal papilla produces a signal that regulates the activity of hair follicle melanocytes and modulates pigment production; as such, the genes involved in hair color are expected to be more diverse than those in the skin. Hair color is one of the most heritable traits, with estimates from twin studies suggesting 97% heritability. Hysi et al. (63) performed a GWAS meta-analysis of almost 300,000 individuals with self-identified European origins from the 23andMe and UK Biobank cohorts. Consistent with a polygenic nature of this trait, they identified more than 120 loci that were significantly associated with hair color, and a striking 107 of the regions were novel; **Table 3** shows a selection of these newly associated genes. The known loci included *OCA2/HERC2* (rs12913832), *IRF4* (rs12203592), and *MC1R* (rs1805007). Several genes involved in melanocyte development were also identified: *EDNRB* (rs1279403), *MITF* (rs9823839), *HPS5* (rs201668750), *FGF5* (rs7681907), *SOX5* (rs9971729), and *TWIST2* (rs11684254). Analysis using Gene Ontology entries for the entire gene set identified a correlation with pigmentation and melanin biosynthetic and metabolic processes. This extensive study explained a significant amount of the heritable variation in hair color: 34.6% for red hair, 24.8% for blond hair, and 26.1% for black hair. Interestingly, it also confirmed a sex bias in hair color, with women demonstrating a preferentially lighter color.

A similar GWAS report for hair color was conducted using a larger cohort of more than 343,000 individuals from the UK Biobank and has extended the number of genes discovered (105). A major focus of this report was the contribution of *MC1R* polymorphism to the red hair phenotype, with SNPs in this locus explaining 73% of the heritability of this trait. The study also found an additional eight associations for red hair at a genome-wide significant level, with statistical fine mapping in some cases indicating a likely causal SNP. Variants in both the *OCA2/HERC2* and *POMC* loci reduced the probability of red hair, with the rs76645354 SNP 2 kb upstream of *POMC* likely to increase expression of the gene that produces the α-MSH ligand for MC1R. The interaction of SNPs around the *ASIP* locus was again shown to influence the red hair phenotype, with rs6059655*G/A being an eQTL for *ASIP* in the skin. The *TSPAN10* gene, highly expressed in melanocytes, has a lead SNP in strong linkage disequilibrium with rs6420484*A/G Y177C for red hair, and this allele has previously been reported to be associated with hue saturation of eye color. Epistatic interactions with the *PKHD1* gene were also found.

This study found that more than 200 genetic variants are associated with hair color on a continuum from black to dark and light brown to blond, with 73% of the heritability of blond hair being explained by these SNPs. The variants identified correspond to 163 distinct genes, 93 of which were also reported by Hysi et al. (63). A polygenic phenotype score developed using these variants confirmed that hair color is a polygenic trait, and many of these hair color loci are enriched as regulatory elements. A major new finding is that seven loci previously associated with hair shape variation—*ERRFI1*, *FRAS1*, *HOXC13*, *PADI3*, *KRTAP*, *PEX14*, and *LGR4*—affect blond versus nonblond hair color. This suggests that genes involved in hair texture that are expressed by keratinocytes may contribute to the determination of hair color.

In addition to baseline skin color, there is a genetic contribution of UV exposure to coloration through the tanning response. Visconti et al. (150) explored the genetics of skin tanning using a GWAS of more than 175,000 people from European ancestries as part of the UK Biobank. Almost 40% of the individuals self-reported their tanning ability as never, mildly, or occasionally.

The authors identified 20 loci associated with tanning ability, six of which were confirmed from previous studies: *SLC45A2* (rs16891982), *IRF4* (rs12203592), *TYR* (rs1126809), *OCA2/HERC2* (rs12913832), *MC1R* (rs369230), and *RALY/ASIP* (rs6059655). The loci included genes previously implicated in pigmentation as well as novel players: *PDE4B* (rs1308048), *RIPK5* (rs12078075), *PA2G4P4* (rs9818780), *PPARGC1B* (rs251464), *AHR/AGR3* (rs117132860), *TRPS1* (rs2737212), *TYRP1* (rs1326797), *BNC2* (rs10810650), *EMX2* (rs35563099), *TPCN2* (rs72917317), *DCT* (rs9561570), *ATP11A* (rs1046793), *SLC24A4* (rs746586), and *KIAA0930* (rs11703668). The mechanisms underlying this complex network of gene interactions are an exciting area for future research.

Several large-scale GWASs for melanoma risk have expanded our understanding of the loci contributing to other pigmentation traits. Duffy et al. (46) performed a meta-analysis of GWASs for the number of acquired melanocytic nevi. The study combined data from populations in Australia, the Netherlands, the United Kingdom, and the United States. The data gathered from more than 52,000 individuals confirmed known loci, including *MTAP*, *PLA2G6*, and *IRF4*, and detected novel SNPs in *KITLG* and in a locus at 9q32. Significant associations were found with SNPs near *GPRC5A*, *CYP1B1*, *PPARGC1B*, *HDAC4*, *FAM208B*, *DOCK8*, and *SYNE2*. Some of these loci also appear in the novel pigmentation loci described above (150).

Conclusions and Perspectives

The development of large databases combining human genotype and phenotype data (22, 51) has allowed GWASs to be performed that herald a new era in understanding the genetic variation in human skin and hair color. While confirming the major genes identified through studies of albinism and comparative genetics (**Tables 1** and **2**), these studies have also provided an expanded list of candidates (**Table 3**) that now need to be investigated at the biochemical, genetic, and population distribution levels (124). It may be possible to incorporate these genes into existing components and networks known to control skin pigmentation (125).

Melanin pigmentation present in the skin is important for photoprotection, and it is remarkable to see the diversity of skin colors that have appeared around the globe following human migration of founder populations combined with environmental selection. Studying the genetic background that determines skin and hair color traits is useful in understanding the process of human evolution and the selective forces that operate to drive such changes. The recent work investigating African populations has indicated a complex genetic landscape of alleles in genes associated with lightening and darkening of the skin (34, 100), and more genes will be identified through the study of pigment variation in Africa. By contrast, European and East Asian populations in high-latitude environments with weak light are subject to a selective force for skin lightening. UV radiation is required for the production of vitamin D, the key nutrient necessary for bone formation, whereas protection from UV is necessary to avoid folate photodegradation, which would select for skin darkening (73). A signature of diversifying selection can now be seen in the major pigmentation genes in Europeans and Asians (62), with the study of new candidate pigmentation genes now required to determine how selection has operated on this expanded list.

The advances made in the past few years in understanding the consequences of polymorphism in the major pigmentation genes and variability among human populations has meant that these causative SNPs can also be used as ancestry-informative markers for biogeographical genealogy studies (121). Moreover, they have allowed the development of predictive models such as HIrisPlex for hair, eye, and skin color in forensic analysis of DNA samples (25, 155). The incorporation of newly identified pigmentation genes into these predictive models will help refine and improve the accuracy with which skin and hair color phenotypes can be determined from an individual's genotype.

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