Skin Pigmentation Genetics for the Clinic

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Abstract
Human pigmentation characteristics play an important role in the effects of sun exposure, skin cancer induction and disease outcomes. Several of the genes most important for this diversity are involved in the regulation and distribution of melanin pigmentation or enzymes involved in melanogenesis itself within the melanocyte cell present in the skin, hair and eyes. The single nucleotide polymorphisms and extended haplotypes within or surrounding these genes have been identified as risk factors for skin cancer, in particular, melanoma. These same polymorphisms have been under selective pressure leading towards lighter pigmentation in Europeans in the last 5,000–20,000 years that have driven the increase in frequency in modern populations. Although pigmentation is a polygenic trait, due to interactive and quantitative gene effects, strong phenotypic associations are readily apparent for these major genes. However, predictive value and utility are increased when considering gene polymorphism interactions. In melanoma, an increased penetrance is found in cases when pigmentation gene risk alleles such as MC1R variants are coincident with mutation of higher-risk melanoma genes including CDKN2A, CDK4 and MITF E318K, demonstrating an interface between the pathways for pigmentation, naevogenesis and melanoma. The clinical phenotypes associated with germline changes in pigmentation and naevogenic genes must be understood by clinicians, and will be of increasing relevance to dermatologists, as genomics is incorporated into the delivery of personalised medicine.

Introduction

The past 2 decades of research on the cellular and genetic basis of human pigmentation has revealed that there is a high degree of variation in genes responsible for colour (amount and type of melanin pigment) and skin type (responsiveness to UV exposure) between and within human populations [1]. Those of European ancestry display the widest variety of skin, hair and eye colours [2, 3]. This review provides a brief history of the evolution of pigmentation genetics in humans and then examines several of the most important genes required for the diversity that occurs in modern human populations. The alleles that render each gene significant are outlined, along with the phenotypic consequences of the causal single nucleotide polymorphisms (SNPs). The biology of pigmented lesions is then discussed, including freckling, naevi and melanoma [4].
Pigmentation Evolution in Humans

There has been a strong selection pressure towards lighter skin, hair and eye colour in European populations in the last 5,000–20,000 years [5]. The origin of modern humans can be traced to Africa around 200,000 years ago, followed by the expansion and subsequent colonization of the rest of the world around 50,000–60,000 years ago.

**Fig. 1.** Measures of pigmentation characteristics in a population of European ancestry. The Brisbane Naevus Morphology Study has used a range of measures for pigmentation phenotype [83] recorded on over 1,200 individuals using digital photography. Examples shown include the following: a Eye colour on a 3-point scale of blue, green-hazel, brown, with and without iris freckling. b Hair colour on a 5-point scale of red, fair-blonde, light brown, dark brown, black with hair greying related to age also recorded. c Constitutional and facultative skin colour on a 3-point scale of fair, medium and olive/dark, also quantitated using skin reflectance. d Freckling using a 4-point index (0–3) on 3 body sites, face, dorsum of the hands, shoulders, giving an overall freckling score (0–9). e Total naevi counts with dermoscopy performed on significant naevi >5.0 mm.
In 2010, DNA was retrieved from 3 Neanderthal bones, which showed strong evidence of a small amount of gene flow to modern humans. Neanderthal ancestry in all present-day non-Africans is estimated to be 1.5–4% [6, 7], and explains a significant proportion of the risk for actinic keratosis lesions from ultraviolet radiation (UVR) exposure [7]. There have been about 135,000 SNPs discovered associated with Neanderthals, affecting lipid metabolism, immunity, digestion, hair and skin [7]. The gene flow most likely occurred at an early stage of the out-of-Africa expansion around 47,000–65,000 years ago, before the divergence of Eurasian groups from each other. Neanderthals lived out of Africa long enough to adapt to higher latitudes with associated climates, diets and pathogenesis [7]. The proto-European ancestral populations expressed darker pigmentation than present populations, which were likely maintained by high UVR levels in low latitudes necessitating protection against UVR-induced damage to DNA, folates and immunoglobulins [5]. Prior to this, earlier species of humans Homo erectus left African rainforests and crossed savannah environments. This migration induced loss of body hair, with increased hair in the head, aiding cooling of the body, but protection of the head from UVR and overheating of the brain. Without hair, the pale body skin darkened to avoid damaging exposure to UVR [8].

Around 11,000–19,000 years ago, the ancestors of modern Europeans and Asians separated, and moves began to areas of higher latitudes with lower UVR levels. Here, the annual dose of UV was not enough for vitamin D3 photosynthesis in darkly pigmented skin. The danger of folate and DNA damage was also reduced [5, 9]. Europe was first populated by modern humans around 45,000 years ago, depopulated during the glacial maximum 25,000 years ago, and subsequently repopulated as the climate improved, with farming beginning ~8,000 years ago during the Neolithic transition [6]. In Europe during the Bronze Age, populations were composed of a mixture of these Neolithic farmers and hunter-gatherers. By examining these ecological drivers of genetic selection, along with frequencies of pigmentation alleles in modern populations, genomic signatures of natural selection can be analysed [5, 10, 11].

During the Bronze Age (1,000–3,000 years BC), there were large amounts of migrations and replacements of populations within Europe and Asia. Before this time (the Iron Age), Asian populations contained more West Eurasian ancestry than today; however, by the end of the Bronze Age, these became similar to the present populations [10, 11]. With the arrival of farming, there was a change in diets, from a vitamin D rich hunting and high protein fishing diet to a low vitamin D, high starch farming diet [5, 12, 13]. In these environments, without oily fish or livers, there was pressure to reduce skin pigmentation for increased vitamin D synthesis. By studying compound haplotype systems within these genes, an attempt has been made to calculate the age of some of the alleles associated with the lightening of European skin colour [3]. The allele age of KITLG has been estimated as 16,480 years, tyrosinase related protein-1 (TYRP1) allele age as 11,930 years [9] and the blue eye colour allele of OCA2 arose approximately 13,000 years ago [14].

**Pigmentation Genetics for Dermatology**

Melanin is a macromolecular biopolymer derived by the oxidation and polymerization of tyrosine [15] that is synthesized in epidermal melanocytes. It is transferred to the surrounding keratinocytes to help protect against the effects of UV radiation (Fig. 2). 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 4 stages. This process includes passaging of the key tyrosinase enzyme (TYR), catalysing 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 yellow/red pheomelanin, with the ratio of each dependent on the catalytic activity of the rate-limiting melanogenic enzyme tyrosinase, and the availability of cysteine. In addition, a number of melanosomal ion transport proteins are critical for melanosomal function [16], and the regulation of melanosomal pH is critical for the process of melanogenesis. Notably, melanosomes from melanocytes of fair-skinned individuals are more acidic and display low tyrosinase activity, whereas melanosomes in dark skin are more neutral and present higher levels of tyrosinase activity.

Dermatologists have always been intimately aware of the clinical implications of differences in skin pigmentation for the consequences of sun-exposure, skin cancer induction and disease outcomes. With such rapid progress in our understanding of the human genome, they may be challenged by the new understanding of the genetics underlying these phenotypes [17] that must be incorporated into the clinic encompassing the move toward personal or precision medicine [18].
Fig. 2. Network of pigmentation gene interaction in the melanocyte and the keratinocyte. The figure is a schematic representation of a melanocyte cell interacting with surrounding keratinocytes of the skin, with the melanosome structure expanded in the upper left of the figure. UVR induces DNA photodamage such as cyclobutane pyrimidine dimer (CPD) formation predominantly in keratinocytes, which activates the p53 protein, leading to POMC, endothelin 1 (EDN1) and KITLG production. The precursor POMC is cleaved to form αMSH, the ligand binding to MC1R; this leads to the activation of the cAMP-PKA-CREB pathway and MITF. In a parallel pathway, KITLG binds to KIT receptor on the melanocyte, leading to the activation of MAPK and then MITF. MC1R action can be blocked by ASIP. MITF and IRF4 lead to the transcription of the melanogenic genes TYR, TYRP1 and DCT. This family of proteins are incorporated into the melanosome inducing eumelanin formation and maturation with transfer to the keratinocyte. Here, melanosomes provide photoprotection from UVR – the tanning response – by protecting the nucleus via a cap. HBD3 is a competitive ligand produced in the keratinocyte that also binds to MC1R. MTAP catalyses phosphorylation of methylthioadenosine (MTA), which blocks the function of cAMP phosphodiesterase (PDE), and hence the cAMP-PKA-CREB pathway and MITF stimulation. In addition, the direct effect of UVR is to induce cell cycle arrest via p16 block of CDK4/6 cyclin proteins in melanocytes. PLA2G6 affects mitochondrial function via membrane phospholipid maintenance (GPL/PL). Inside the melanosome, TYR and cysteine availability affect the formation of the type and density of melanin formed within the melanosome. MATP and NCKXS work as ion transport proteins, facilitating the functions of the melanogenic enzymes. Finally, OCA2 can act at multiple levels to influence the melanosome, the melanogenic enzymes, pH and glutathione metabolism.
Pigmentation is a polygenic trait due to interactive and quantitative gene effects, with polymorphisms in several major genes involved in producing the continuous range of pigmentation phenotypes apparent between and among different ethnic groups [3, 19, 20]. Recently, genome wide association studies (GWAS) have recognised the contribution of several of these loci involved in the pigmentation pathway with the risk of squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanoma [21–25]. These include some of the 10 major pigmentation genes (Table 1), which are discussed below, together with 5 additional genes highly relevant to naevi and melanoma [15].

**MC1R: α-MSH Receptor and the Gene for Red Hair Colour**

One of the most important systems regulating human pigmentation is the G-protein coupled α-MSH receptor MC1R, expressed on the cell surface of the melanocyte, commonly known as the gene for the red hair colour (RHC) phenotype. Population-based studies in diverse ethnic groups have shown the MC1R gene to be highly polymorphic with around 200 allelic variants identified [26, 27], 9 common variants are recognised in populations with European ancestry [28]. In Caucasians, some of the MC1R variants known as R-alleles, with a combined frequency of 18%, have a strong association with red hair, fair skin, freckling as well as increased melanoma and non-melanoma skin cancer risk. The most common R variant alleles are D84E, R142H, R151C, R160W and D294H [26, 29]. MC1R alleles less penetrant for RHC are known as r-alleles of 27%, with the normal wild type allele (WT) at around 50% frequency. The common r-alleles are V60L, V92M, I155T, and R163Q. It is now recognized that MC1R has effects that extend beyond pigmentation, and involve the activation of the DNA damage response in human melanocytes. This role of MC1R is pivotal for the prevention of photo-carcinogenesis; it displays MC1R function as a melanoma predisposition gene and reveals how the expression of RHC variants increases melanoma risk [26, 27].

The ratio of the 2 types of melanin is dependent on the catalytic activity of the rate-limiting melanogenic enzyme tyrosinase, and the availability of cysteine. High tyrosinase activity and/or low concentrations of cysteine lead to the synthesis of the photoprotective eumelanins [30]. Conversely, low tyrosinase activity and high availability of cysteine lead to the less photoprotective and potentially phototoxic pheomelansins [31]. The main determinant of the amount and type of pigments formed within epidermal melanocytes is the MC1R genotype status [26, 28].

Upon stimulation by its endogenous agonists, α-MSH or ACTH, which bind with equal affinity, the Ga subunit interacting with the MC1R protein is activated, followed by adenylate cyclase, leading to increased cAMP formation. Protein kinase A (PKA) is then activated, followed by the phosphorylation of members of the cAMP responsive-element binding protein (CREB) family of transcription factors, and CREB-mediated activation of Microphthalmia transcription factor (MITF) gene expression. MITF is a key positive regulator of melanocyte differentiation markers including tyrosinase activity, increased protein levels of TYRPI and dopachrome tautomerase (DCT), leading to the biosynthesis of eumelanin pigments [26, 28].

RHC as a recessive trait requires carrying 2 R variants in the homozygous or compound heterozygous state. Three of the most common R variants important in skin type and freckling are MC1R R151C, R160W and D294H. The variants found to result in reduced cAMP signaling were V60L, D84E, R142H, R151C, I155T, R160W and D294H [29], that are significantly associated with melanoma development [27, 32]. This was observed by an abolished response to α-MSH in melanocytes homozygous for RHC variant alleles, and an absence of reduction in UV-induced hydrogen peroxide generation or enhancement of repair of DNA photoproducts [33]. Large differences in the distribution of MC1R variants across European populations have been observed. For example, V60L is at >20% allele frequency in Italy, but around 12% in Northern Europe, where R151C is >10%, but is <5% in South European Populations [34].

**Agouti Signaling Protein: An Inhibitor of MC1R and Pigmentation**

Agouti signaling protein (ASIP) locus on chromosome 20q11 is implicated by genome-wide association studies in phenotype variation and melanoma risk [35, 36]. The ASIP gene product is a 131 amino acid protein that antagonizes the interaction between MC1R and α-MSH, facilitating pheomelanin production. The association of melanoma with the ASIP region closely parallels that with RHC, and seems to be largely due to a single long (~1.8 Mb) haplotype containing 22 known genes including ASIP [20, 35, 37]. The ASIP haplotype identified is tagged by markers rs1015362*G and rs4911414*T, located over
### Table 1. Human pigmentation and naevogenc genes with phenotype associations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>SNP</th>
<th>Codon/position/haplotype</th>
<th>Amino acid/isoform</th>
<th>European allele frequency, %</th>
<th>Skin</th>
<th>Eye</th>
<th>Hair</th>
<th>Naevi</th>
<th>Freckling</th>
<th>Albimism/genetic disease</th>
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<td><strong>TYR</strong></td>
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<td>Ser192Tyr</td>
<td>63.37/36.63</td>
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<td>rs1126809/G/A</td>
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<td>Arg402Gln</td>
<td>73.52/26.48</td>
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<td>5' distal/HERC2 intron86</td>
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<td>A-A-A-G</td>
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</table>


100 kb upstream of the ASIP gene but strongly associated with melanoma [38], BCC risk and pigmentation. Similar to the RHC variants, the ASIP haplotype associates with freckling, skin sensitivity to sun, and red and blonde hair [20]. Another polymorphism rs4911442*T/C, located within intron 5 of the nuclear receptor coactivator 6 (NCOA6) gene and down-stream of ASIP, was also reported to be strongly associated with melanoma [36]. The
rs4911442*C allele was equivalent to an “r” MC1R allele suggesting a gene–gene interaction between MC1R and the rs4911442 locus on the effect of hair colour and melanoma [20].

**TYR (OCA1): The First Gene in the Pigmentation Pathway and Responsible for Albinism Type I**

TYR was one of the first human pigmentation genes identified, with reports showing it to be mutated in OCA1 albinism and accounting for 46% of cases of albinism in European populations [39]. It is the enzyme that catalyses the first step required for melanin pigment production, the oxidation of its substrates tyrosine and DOPA to form the intermediate DOPAquinone. More than 100 mutations of this gene have been identified [40, 41]. Two common polymorphisms, rs1042602*C/A S192Y in exon 1 and rs1126809*G/A R402Q in exon 4, appear at a high frequency in Europeans and are largely absent in African populations [3, 42]. The S192Y allele is associated with light skin, eye colour, absence of freckles and an increased SCC risk, whereas the R402Q change was reported to increase the risk of BCC [43], was frequently associated with albinism patients [44] and significantly increased SK, multiple melanoma risk [42] and familial CM risk in a French population [41]. Protein expression studies have shown that the 402Q variant encodes a thermolabile enzyme [40], and in primary melanocyte cultures we have shown that it is retained in the endoplasmic reticulum, hypoglycosylated and preferentially degraded [45]. Interestingly, thermolabile TYR alleles are the basis of the Siamese/Burmese cat coat colours, where pigment appears darkest at the extremities of the body [46]; this condition is also present in some human albino patients as subtype OCA1B [39]. Recent studies have shown that the TYR genotype is likely to be a significant modifier of other pigmentation gene polymorphisms in human skin, hair and eye colour, and associated with naevus count, though not apparent by body site there is potential for thermal changes in pigmentation of the skin and hair [45]. The 2 polymorphisms were present on 4 TYR haplotypes, designated as WT being 192S-402R, single variants 192Y-402R and 192S-402Q, with a double-variant 192Y-402Q of low frequency at 1.9%. We have used an additive model to assess the penetrance of the 10 possible TYR genotypes derived from the combination of these haplotypes. The double-variant 192Y-402Q haplotype is likely to be deleterious, and may explain the association of the 402Q SNP with albinism and hypopigmentation in the general population [45].

**OCA2: The Major Gene for Eye Colour and Albinism Type II**

OCA2 encodes the P-protein, which assists tyrosinase trafficking and processing [47], melanosomal pH and glutathione metabolism [48]. It has recently been shown to assist in anion transport increasing chloride conductance from the melanosome [49]. The R305W (rs1800401), R419Q (rs1800407), and V443I (rs1219841) coding polymorphisms are associated with eye colour [50, 51] and R419Q is associated with increased BCC risk [43]. The A481T (rs74653330) and H615R (rs1800414) alleles are more common among Asian populations and associated with skin lightening [52].

Human eye colour is a highly variable phenotype under strong genetic control. GWAS studies have shown the OCA2 locus, which is the gene responsible for oculocutaneous albinism type II when mutated, as the likely major gene influencing blue-brown eye colour. The key determinant SNP rs12913832*T/C located within intron 86 of HERC2, 21kb upstream from the OCA2 initiation site showed the strongest association with blue-brown eye colour. The rs12913832*C allele is highly associated with blue eye colour, and occurs at a frequency of 69%, and rs12913832*T associated with brown eye colour occurring at a frequency of 31% [2, 51]. Examination of genotypes derived from skeletons in several archeological sites has revealed that some pre-European populations had dark skin and hair pigmentation, with the rs12913832*C SNP in HERC2/OCA2 leading to blue eye colour, 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 [10, 12]. Proportionate lightening when carrying recessive blue-eyed OCA2 and red-hair R alleles indicates additive action of MC1R and OCA2 loci on constitutive skin colour [53]. The modifying effect of OCA2 on MC1R is also seen for freckling score; blue eye colour increases freckling, and the R allele further increase the freckling score in an additive fashion [53, 54]. The frequencies of rs12913832 genotypes were 62.5% blue-eyed children homozygous for the C allele, and 89.8% brown-eyed children T allele homozygous. In a study of childhood sun exposure habits, the blue-eyed rs12913832*C allele interacted with MC1R RHC to increase sunburns and naevi numbers over 2 mm [55].
**TYRP1 (OCA3): Albinism Type III**

The *TYRP1* gene also encodes a protein involved in the melanogenic pathway [56], with complete loss of function *TYRP1* mutations leading to OCA3 or rufous albinism [39, 44]. *TYRP1* can act to stabilise the TYR protein and form heterodimeric complexes within the melanosome [57]. It was found that 95% of OCA3-related mutations result in the generation of premature stop codons or frameshifts producing truncated proteins, red-toned hair, reddish-brown pigmented skin and optical features not as severe as in other forms of OCA [58]. Only 5 mutations have been described so far in *TYRP1* [39]. *TYRP1* plays a role in the eumelanin pathway, giving rise to black-brown pigmentation (eumelanin), which occurs at high levels in individuals of African and Oceanic descent. A single SNP, rs1408799, showed genome-wide significant association with blue versus non-blue eyes in Icelandic and Dutch samples. A suggestive association with blonde versus brown hair was also observed for this SNP [37]. A C93R amino acid change at a highly conserved residue in *TYRP1* was discovered as a source of blonde hair in Solomon Islanders [59]. The novel mutation rs387907171*C/T occurred at a frequency of 26% in the Solomon Islands but was absent outside Oceania. In contrast, *TYRP1* was excluded as being the gene responsible for a form of OCA in a Polynesian population in another study [58]. It is notable that at the same position there is a C93H polymorphism present at 1.1% frequency in the European population [60], but an association with a pigmentation phenotype is yet to be examined.

**DCT: An Early Marker of Melanocytes and Protective Response to UV**

The melanogenic enzyme *DCT* (originally known as the tyrosinase related protein-2, TYRP2) is involved in the formation of the photoprotective skin pigment eumelanin by the isomerization of the red-yellow intermediate dopachrome to 5,6-dihydroxyindole-2-carboxylic acid. This precursor is oxidized into the eumelanin pigment along with 5,6-dihydroxyindole, providing protection against the damaging effects of UVR in the skin. However, *DCT* has also been shown to have a role in the melanogenic cell response to apoptotic stimuli and oxidative stress [61] 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 in Asians, but only scarce signals in Africans [62, 63]. However, the overall picture reflects a complex pattern of selection, which might include over-dominance for *DCT* in Europeans [63]. Analysis of the 1,000-genome data [1] confirms the existence of several non-coding region polymorphisms with higher-than-expected heterozygosity in the extended *DCT* region in Europeans [63]. The SNPs rs1407995*T/C and rs2031526*A/G are *DCT* intronic polymorphisms each showing frequencies of 13.64% in East Asian populations indicative of linkage. The effects of these SNPs on pigmentation phenotypes are at this stage uncertain, but natural variations in *DCT* expression lead us to suspect that *DCT* interacts with other pigmentation genes [64].

**SLC45A2 (OCA4) and SLC24A5 (OCA6): The Major Genes for Skin Colour Variation**

A polymorphism having a significant effect on skin and hair colour is the rs16891982*G/C SNP within the *SLC45A2* gene, which results in a missense amino acid change L374F in the MATP protein. The *SLC45A2* gene is expressed at high levels in melanocytes and melanomas, with the MATP protein located in melanosomes [65]. 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 [66]. In contrast, the 374F variant is predominant at a frequency of over 95% in the light skinned European populations, and can be predicted to impair the MATP function [67]. Another polymorphism significantly associated with skin and hair colour is rs1426654*G/A identified in the *SLC24A5* gene, encoding the NCKX5 protein change A111T, with a European population frequency of 111T near 99%. The 111T allele frequency was found to increase in Europeans in the 3,000 years between the Mesolithic and Bronze Age, while the 111A ancestral allele is again close to fixation in dark-skinned African populations [2, 10]. Both MATP and NCKX5 are membrane bound ion transport proteins (Fig. 2), implying that the active pumping of ions within the melanosome is crucial to the production of melanin [67].

In addition to the genetic associations of *SLC45A2* and *SLC24A5* alleles, their melanogenic activities have been examined in clonal human melanocyte cell cultures of defined genotypes. On average, MATP-374L/L homozygote strains were found to have 2.6-fold higher melanin content and a 2.8-fold higher tyrosinase activity than European MATP-374F/F strains, while NCKX5−111A/A
strains had 2.2-fold higher melanin content and a 1.7-fold higher tyrosinase activity than NCKX5–111T/T [66].

Mutation of SLC45A2 leads to OCA4 [68]. It is known that MATP abrogation leads to lowered melanosomal pH, and reduced tyrosinase activity, possibly due to its effects on copper binding to tyrosinase protein. This leads to immature, weakly melanised melanosomes [65]. The mutation of SLC24A5 leads to OCA6 that can impair or disrupt melanosomal maturation, and therefore normal melanin biosynthesis. This leads to lighter hair colour that darkens with age, iris trans-illumination, and reduced visual acuity. OCA6 is one of the rarest forms of OCA [69].

**KITLG: A Gene for Blonde Hair Colour**

Piebaldism, an autosomal dominant disorder caused by altered melanocyte proliferation and migration presenting as white spotting patches of the skin or hair, has been associated with mutations in the c-KIT gene [70]. Normal variation in this gene is seen, such as rs3822214*A/C M541L is around 9.7% allele frequency [1, 60], but there are no population-specific or phenotypic associations seen with this allele. The ligand for the KIT receptor KITLG is known to regulate the number of melanocytes during development and melanin distribution in the skin; it is also known to activate keratinocytes to produce promelanogenic factors and keratinocyte growth factors to promote melanosome phagocytosis and activate the onset of familial progressive syndromes of both hyper- and hypo-pigmentation [70, 71]. Histologically, hyper-pigmented areas exhibit normal epidermis with strongly hyperpigmented basal keratinocytes and melanophages in the upper dermis. The absence of systemic symptoms and signs suggests a pathogenesis related to melanogenesis. A transversion (c.107A>G) in exon 2 of KITLG is responsible for inherited familial progressive hyperpigmentation, producing a gain-of-function defect in tyrosinase activity and melanin synthesis [70].

The first indication that polymorphism of KITLG could be associated with human skin colour was the report that rs642742*T/C was associated with a higher melanin index in an African-American population. The non-coding rs12821256*T/C SNP located in a large intergenic region over 350 kb upstream of the KITLG transcription start site alters the binding site for the lymphoid enhancer-binding factor (LEF) transcription factor. This reduces LEF responsiveness and enhances activity in cultured human keratinocytes [72], impacting the expression of the gene. The SNP rs12821256 is genetically associated with blonde hair in northern European populations such as Iceland and the Netherlands at a frequency of 13% [3, 71–74].

**IRF4: A Gene for Tanning and Naevi**

IRF4 is a member of the interferon regulatory factor family of transcription factors, not previously known to play a role in melanocyte biology before the discovery of the rs12203592*C/T SNP located in the fourth intron of the IRF4 gene. This rs12203592*T polymorphism was found to be strongly associated with darker hair colour, hair greying, lighter eye colour and reduced skin tanning response to sunlight [19, 73, 75] and it lies within an enhancer element that drives IRF4 transcription in melanocytes. The rs12203592*T allele impairs binding of the TFAP2A transcription factor that, together with the melanocyte master regulator MITF, regulates the activity of the enhancer [76]. It has also been found that MITF directly or indirectly regulates IRF4 expression, and that TYR expression depends on IRF4 protein [76]. Asian and African populations are fixed for the rs12203592*C allele, being monoallelic, with only European populations occupying the rs12203592*T at around 17% allele frequency. Homozygous carriers of rs12203592*T show higher naevi counts as adolescents, which reverses over age with adults having lower naevi counts than those that are heterozygous or homozygous rs12203592*C [77–79]. The rs12203592*T allele was positively associated with melanoma and solar elastosis, and statistically and significantly associated with having 10 or fewer back naevi, dark hair colour, light eye colour and decreased ability to tan [80]. European populations with the IRF4 rs12203592*C/C genotype had predominantly an early onset of melanoma distribution, peaking around the age of 45, while patients with the rs12203592*T/T genotype had a predominantly late-onset distribution, peaking around age 75 [80].

**MITF: The Master Regulator of Melanocytes with Major Effects on Naevi and Melanoma**

A germline missense substitution rs149617956*G/A of the microphthalmia transcription factor MITF gene encoding a SUMOylation-deficient E318K-mutated protein has been described as a medium-penetrance melanoma gene occurring at a frequency of around 2.1% dependent upon European population examined [81, 82]. MITF
E318K carriers had a reported two- to fivefold increased risk of developing melanoma. The MITF 318K allele encodes a protein that biochemically impairs MITF SUMOylation at position 316K that led to differential regulation of several MITF targets. MITF E318K enhanced MITF protein binding to the HIF1A promoter and increased its transcriptional activity compared to WT MITF, and enhanced melanocytic clonogenicity, migration and invasion [81]. Carrying this variant is genetically associated with increased naevus number, risk for melanoma, and higher incidence of multiple primary melanomas, being significantly over-represented in cases with a family history of melanoma [82] and/or renal cell carcinoma [81]. Generally, the study phenotype consisted of fair skin, freckling and a high total naevus count. A high incidence of amelanotic melanomas was found, with 3 of the 5 melanomas from one MITF E318K and MC1R RHC variant genotype patient, suggesting a genetic interaction between these 2 genes [83].

**MTAP: A Gene for Naevi and Melanoma**

The MTAP gene is located on chromosome band 9p21, adjacent to the familial melanoma susceptibility locus, CDKN2A [84]. MTAP was found to associate strongly with naevus count in European-derived populations in Australia and the United Kingdom, and was significantly associated with melanoma risk in these populations. An MTAP tagging haplotype consisting of 4 SNPs, rs4636294*A/G SNP located in the 5’ UTR of MTAP, rs935055*G/C and rs7023329*A/G in intronic regions of the gene and rs7023954*G/A a coding change V56I, are associated with this increase in adult naevus counts and melanoma [84–86]. The A-G-A-G and G-C-G-A protective haplotypes are at approximately equal frequencies in European populations. Notably, MTAP is often co-deleted with the adjacent melanoma susceptibility locus CDKN2A. The loss of MTAP in some tumours has generally been attributed to its proximity to CDKN2A but further studies suggest MTAP functions as an independent tumour suppressor. MTAP encodes an enzyme that catalyses the phosphorylation of methylthioadenosine, a by-product of the synthesis of polyamines, and cultured MTAP-deficient cells are known to secrete methylthioadenosine, which can inhibit melanoma cell line growth (Fig. 2). In addition, the secretion of methylthioadenosine has a variety of effects on surrounding cells that can influence tumour progression [87]. The cellular vulnerabilities created by MTAP loss have been investigated [88, 89], these studies have shown a selective dependence on PRMT5 gene expression, a histone methyltransferase, for viability in MTAP-deleted cell lines. Moreover, quantification of metabolite levels in MTAP-proficient and MTAP-deficient cells indicated that enzymatic loss of MTAP increased the abundance of methylthioadenosine, and this accumulation suppressed PRMT5 activity.

**PLA2G6: A Gene for Naevi and Melanoma**

Phospholipase A2 (PLA2G6) belongs to the superfamily of genes, which encode esterases that cleave glycerophospholipids (Fig. 2). These enzymes are normally involved in the maintenance of membrane phospholipids, but have recently been shown to regulate cell growth, apoptosis, and cell proliferation in human tumours [84]. An extended haplotype around PLA2G6 consisting of the intronic SNPs rs11570734*A/G, rs2284063*AA/G, rs6001027*A/G, and rs4384*G/C is associated with increased melanoma risk and naevus counts [84, 86]. The A-A-A-G is the major and risk-associated haplotype at a frequency of over 55%. Notably, the combined additive effect of the PLA2G6 rs2284063*A and rs4636294*A in the MTAP gene markedly increases the observed number of naevi [84]. In a more recent study, multivariate analyses showed that the minor alleles of IRF4 rs12203592*T and PLA2G6 rs738322*A were significantly associated with increased naevus count [78].

**CDKN2A/CDK4: Familial Melanoma Genes of Low Frequency**

The major susceptibility locus for melanoma is CDKN2A on chromosome 9p [90]. About 5–10% of melanoma is associated with familial predisposition with affected first- or second-degree relatives. Of those, 20–40% familial melanoma is linked to chromosome 9p21 locus and a proportion of 9p21-linked families carry disease-segregating germline mutations in the cyclin-dependent kinase inhibitor 2A CDKN2A gene [91–93]. The majority of mutations at this locus are single base pair substitutions in exons 1a and 2, affecting the function of the protein p16/INK4A. Some of the mutations in exon 2 also impact on the alternative splice product of the locus, p14ARF [90]. Variants in CDKN2A confer very high risk, but are limited to a small fraction of the general melanoma population. An early study found the strongest evi-
dence for linkage of counts for flat naevi at the region of the CDKN2A gene [94]. Another early study found that a CDKN2A mutation in the presence of a homozygous consensus MC1R genotype had a raw penetrance of 50%, with a mean age of 58.1 at onset. When an MC1R variant allele was also present, the raw penetrance of the CDKN2A mutation increased to 84%, with a mean age of 37.8 at onset [91] (Table 2). The prevalence of CDKN2A among individuals with a single primary melanoma was reported as 1.2%, while the rate of germline CDKN2A mutations among people with multiple primary melanomas was 5–15%. The overall penetrance of CDKN2A mutations was calculated to be 30% by 50 years and 67% by 80 years. However, analyzing by the geographic location, by 50 years, penetrance was 13% in Europe, 50% in the United States, and 32% in Australia; but by 80 years of age, it was 58% in Europe, 76% in the United States, and 91% in Australia [92].

The CDK4 or CDK6 proteins along with cyclin D control passage through the G1 checkpoint of the cell cycle; these CDKs are selectively inhibited by p16/INK4A (Fig. 2). CDK4 gene mutations that fall within the p16-binding region create oncogenic variants resistant to physiologic inhibition by p16/INK4A. Both somatic and germline mutations of CDK4 have been identified in melanoma cells and in families. Mutations within the CDK4 gene are even less frequent than those within CDKN2A [95]. The germline Arg24Cys mutation in CDK4 generates a dominant oncogene that is resistant to normal physiological inhibition by p16/INK4A in the senescence pathway [96], and has been used successfully to culture naevocytes, overcoming their arrested growth [97]. Notably, a higher frequency of MC1R RHC alleles has also been reported in CDK4 gene mutation melanoma-prone family members with multiple primary melanomas [95, 98].

### Table 2. Effect of combined MC1R RHC genotype and CDKN2A mutation status on melanoma risk and age at onset

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MC1R RHC</th>
<th>CDKN2A</th>
<th>CMM+</th>
<th>CMM–</th>
<th>Penetrance</th>
<th>Mean age at onset (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT WT</td>
<td>0</td>
<td>10</td>
<td>nil</td>
<td>82.4 (3.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant WT</td>
<td>7</td>
<td>38</td>
<td>15.6</td>
<td>58.1 (7.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT mutation</td>
<td>5</td>
<td>5</td>
<td>50</td>
<td>37.8 (1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant mutation</td>
<td>57</td>
<td>11</td>
<td>83.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From a sample of 15 familial melanoma pedigrees [86]. *a Three common MC1R RHC alleles R151C, R160W, D294H. *c Mutations in the heterozygous state.

### Interaction of Gene Variants in Skin Pigmentation, Naevi and Melanoma

#### Gene Variants in Pigmentation

Skin pigmentation is classified as either constitutive or facultative. Constitutive pigmentation is the basal melanin production without external environmental stimuli, while facultative pigmentation represents enhanced melanin production due to sun exposure, UVR or endocrine stress. Constitutive pigmentation is measured via skin complexion, natural hair and eye colour and freckles, while facultative pigmentation is sometimes measured via the Fitzpatrick skin type [43]. Several pigmentation genes have been observed in the process of evolutionary genetics, and are of importance for the pathogenesis of skin cancers [1, 5, 43]. Some of those most associated with these traits include MC1R RHC alleles, ASIP AH haplotype, TYR, HERC2/OCA2, SLC45A2 and SLC24A5. Assay for these highly penetrant SNPs determining a person’s skin phenotype will in the near future enter the clinic and provide additional information for identifying at risk patients for skin cancer screening than relying on the observed or self-reported skin phenotypic characteristics.

Gene variants in SLC45A2 in Asians and MC1R in Europeans have been associated with solar lentigines, which constitute pigmented spots found on photodamaged skin, as well as seborrheic keratosis. Specifically, IRF4, MC1R, ASIP, and a newly identified gene Basonuclin 2 (BNC2) rs62543565*A/C variants contribute to facial ageing pigment changes via pathways independent of basal melanin production [99].

#### Ephelides and Actinic Lentigines

Freckles, the lay term for ephelides, are benign, flat macules affected by sunlight. Ephelides are largely genetically determined small pigmented spots red to light
brown in colour generally 1–2 mm induced by sunlight observed in fair-skinned and/or red-haired individuals that first appear at the age of 2–3 years, then increase during adolescence and often partially disappear with age. They are most frequently found on the face, arms, neck and chest and become more pigmented during summertime. Actinic lentigines (AL) are solar lentigines, inelegantly also called lentigo senilis, sunburn freckles, freckles in adulthood, aging spots, liver spots, generally larger, ranging in size from millimeters to centimeters, light to dark brown and induced by sun exposure and photo-damage of the skin mainly in Caucasians and Asians. They are more common after 50 years on the face, hands and forearm, and their pigmentation characteristics are less affected by the seasons [79].

Ephelides have been found to contain highly pigmented melanocytes with multiple large melanosomes characteristic of dark-skinned individuals, whereas non-freckled areas contain fewer, smaller and less-pigmented granules. ALs have increased numbers of keratinocytes with accumulated melanin from the surrounding melanocytes in the epidermis, with increased TYR expression in the melanocytes. Thus, the number of melanocyte precursor cells is already higher in SLs and each melanocyte is more active. Contrary to ephelides, SL melanosomes are of normal size as well as in melanocytes of adjacent regions. But melanocytes in SLs have more mitochondria and a better-developed endoplasmic reticulum [79].

A number of genes have been shown to be important for the formation of freckles, including MC1R, IRF4, ASIP, TYR, BNC2 [79] and OCA2 [55]. These benign, pigmented spots were observed on sun-exposed skin of fair or RHC children 1–2 mm in diameter [55]. Indeed, the MC1R gene has been shown to be a major contributor to the formation of freckles. The exact function of BNC2 is presently unknown.

### Naevi and Melanoma

Pigmented lesions may first appear as a benign naevus commonly small, symmetrical and quiescent [100]. These naevi can progress to a dysplastic naevus, which is larger, containing some cellular atypia. Melanomas may display at first a radial growth phase, an expanding thin lesion restricted to the epidermis (in situ melanoma), before progressing to a vertical growth phase with large dividing nests of cells in the dermis, before the development of metastatic melanoma occurs, spreading to other areas of the body [101–103]. Patients with multiple dysplastic naevi are at higher risk of developing melanoma. An individual with a single clinically dysplastic naevus has a twofold increased risk for melanoma, whereas having greater than 10 clinically dysplastic naevi is associated with a 12-fold increased risk [104]. For each additional naevus on the body, relative risk of CMM increases by 3% [105] and newly published work indicates that over 50% of CMM arise from naevi [106], but this remains to be confirmed in larger studies. Dysplastic naevi are more common in patients with melanoma, and a personal and family history of melanoma can significantly increase the incidence of finding dysplastic naevi in a patient. When dysplastic naevi were separately analyzed, 57.9% were positive for the now diagnostic BRAF gene mutation [101, 102].

Both chronic and acute sun exposure contribute to total naevi counts. Children with the highest chronic exposure had the highest naevi counts. Naevi size and counts have been related to the OCA2/HERC2 genotype, but not to MC1R R/r or R/R variants [55]. Increased numbers of naevi and large naevi were associated with holiday sun exposure, particularly on intermittently sun-exposed body sites. SNPs in MTAP and PLA2G6 were associated with increased numbers of naevi and larger naevi, whereas IRF4 was associated only with large naevi. Melanoma risk was associated with increased naevus count, large naevi and atypical naevi for tumours in all body sites (including rare sites) irrespective of age. The risk persisted when adjusted for inheritance of naevus SNPs [107].

### Conclusions

Although there are many genes involved in human melanogenesis, the major genes with significant effect for skin, hair and eye colour, freckling and naevogenesis have now been identified. In this review, we discussed 15 genes with common alleles or haplotypes influencing these interrelated phenotypes (Table 1). A few of these have high penetrance for each of these traits, the exception being TYR, the enzyme that is critical for and the transcription factor IRF4 most required for melanin synthesis. In contrast, some genes appear to have large effects for only 1 or 2 tissues such as OCA2 for eye, MC1R for hair and skin, and SLC45A2/SLC24A5 for skin colour. The list of genes and polymorphisms with significant associations will no doubt grow in the coming years and be important for a full understanding of an individual’s phenotype, including naevus count and skin cancer risk. The increased pen-
etrance of CMM found in familial melanoma families when pigmentation gene risk alleles such as \(MC1R\) RHC alleles are coincident with the mutation of high-risk genes such as \(CDKN2A\), \(CDK4\) and \(MITF\) E318K, demonstrates the interaction between the pathways for pigmentation, naeogenesis and melanoma. The occurrence of these germline mutations within the melanocytes of the skin will be dependent both directly and indirectly on an individual’s pigmentation genotype [108] and the alleles so far shown in Table 1 must be considered and actively incorporated into clinical practise.

**Key Message**

The influence of common polymorphism in pigmentation genes determining skin type must be understood by dermatologists.

**Disclosure Statement**

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