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REVIEW ARTICLE

Genetic risk factors for melanoma

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Abstract The genetic basis of melanoma is complex and has both inherited and acquired components. Different genomic approaches have been used to identify a number of inherited risk factors, which can be stratified by penetrance and prevalence. Rare high-penetrance factors are expressed in familial clustering of melanoma and include mutations in *CDKN2A* (encoding $p16^{INK4a}$ and $p14^{ARF}$) and *CDK4*. These genes are involved in cell-cycle arrest and melanocyte senescence and are nearly invariably targeted by somatic mutations during melanoma progression. Low-penetrance factors are common in the general population and include single-nucleotide polymorphisms in or near *MC1R*, *ASIP*, *TYR* and *TYRP1*. These genes are major determinants of hair and skin pigmentation, but their role in melanoma development remains unclear. This review describes the efforts that have led to the current understanding of melanoma susceptibility as the result of complex gene–gene and gene–environment interactions. Despite the significant advances, the majority of familial cases remain unaccounted for.

Introduction

The incidence of malignant melanoma has increased at a disturbingly rapid pace over the last 50 years. From being a rare cancer a century ago, the average lifetime risk for melanoma has now reached 1 in 50 in many western populations (Giblin and Thomas 2007). Due to a high mortality rate of metastatic disease and a relatively high incidence among adolescents and young adults, melanoma has become a cancer with a major socioeconomic impact (Tsao et al. 2004a). A part of the dramatic increase in melanoma incidence can be ascribed to increased exposure to ultraviolet radiation (UVR), the only known environmental risk factor in melanoma (Tucker and Goldstein 2003). However, intense research has unraveled a complex web of inherited and stochastic etiological factors. Many of these additional factors have been identified as genomic changes, which can either arise de novo, in individual melanocytic cells, or be transmitted in the germline from one generation to the next.

The public availability of the complete human genome sequence and the tremendous advances in technological platforms for genome analysis have boosted melanoma research. A variety of approaches, including linkage analysis, positional cloning, sequencing of candidate genes and genome-wide association studies (GWAS), have revealed a number of gene variants that predispose to melanoma development. The purpose of this review is to provide an overview of these variants and discuss how they may interact with environmental exposures and co-operate with somatic mutations in melanomagenesis.

The molecular basis of melanoma development

Melanoma originates in melanocytes, a specialized cell type whose major function is to produce the melanin pigments that determine skin, hair and eye color (Lin and Fisher 2007). During embryogenesis, melanocytic precursor cells emerge in the neural crest from where they migrate to populate various sites, including the epidermis, the hair follicles, the uveal tract of the eye and the cochlea (Tolleson 2005). It is still unknown whether the melanoma-initiating

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cell is the differentiated melanocyte or an undifferentiated melanocyte precursor cell, or both (Schatton and Frank 2008). In any event, the development of melanoma from a normal cell takes place as a sequence of acquired alterations in specific genes (Fig. 1).

Extensive genome analysis using a variety of technologies, including high-throughput sequencing and array systems, has revealed numerous differences between the genomes of melanoma cells and the normal melanocyte genome. These alterations can broadly be categorized as genetic (mutations, deletions, amplifications and translocations) and epigenetic (promoter hypermethylation). However, only a minority of the alterations found in individual tumors provide the cells with a significant growth advantage over their normal counterparts. These alterations are denoted "driver" events as they represent driving forces in tumor development, although they may not necessarily be required for the maintenance of the final cancer. The remaining genome alterations represent a heterogeneous collection of changes with little or no phenotypic effect, which are collectively known as "passenger" events. Below, we briefly summarize the known melanoma drivers and the sequence of events during melanoma development. It should be emphasized that the proposed model (depicted in Fig. 1) is simplistic and partly hypothetical, and that melanoma development may not necessarily take place along a linear progression line as indicated. Mutations in some of the genes involved in melanomagenesis may also be inherited in the germline and constitute predisposing factors (Fig. 1). The genome alterations and molecular pathways involved in melanoma development have been described in detail in several review articles (Bennett 2008a; Chin et al. 2006; Dahl and Guldberg 2007; de Snoo and Hayward 2005; Miller and Mihm 2006).

Activation of the MAPK pathway

A major achievement in melanoma research was the demonstration of mutations in *BRAF* in a large proportion of melanomas (Davies et al. 2002). This gene encodes a kinase that acts in the mitogen-activated protein kinase (MAPK) signal transduction pathway and thus is important for regulation of a variety of cellular processes, including growth, survival and migration (Wellbrock et al. 2004). Oncogenic *BRAF* mutations lead to constitutive activation of the kinase activity of BRAF, which provides the cell with continuous growth signals in the absence of extracellular stimuli (Wan et al. 2004). Somatic *BRAF* mutations have been found in 60–70% of all melanomas, with a single substitution (p.V600E) accounting for >90% of all *BRAF* mutations (Platz et al. 2007). Notably, this mutation has also been found in up to 80% of benign and dysplastic nevi (Pollock et al. 2003). Although this would suggest that mutation of *BRAF* represents one of the earliest events in melanomagenesis, data from other studies indicate that *BRAF* mutations are acquired as melanomas progress from an in situ to an invasive stage (Greene et al. 2009).

Melanomas without *BRAF* mutations usually carry mutations in other components of the MAPK pathway. The most commonly mutated oncogenes include *NRAS* (Platz et al. 2007), *KIT* (Curtin et al. 2006a) and *GNAQ* (Van Raamsdonk et al. 2009). The distribution of these mutations is not random but vary according to the histological subtype and body site location. *BRAF* and *NRAS* mutations are found in the majority of melanomas of the skin, *KIT* mutations are frequent in acral melanomas of the soles and palms and mucosal membranes, and *GNAQ* mutations are found in uveal melanomas and blue nevi. In addition, *BRAF* mutations are frequent in melanomas on intermittently sun-exposed skin, but infrequent in melanomas on sun-protected body sites (Platz et al. 2007). The biological basis of this mutation distribution remains unknown, but may reflect differences in intracellular signaling and microenvironment.

Inactivation of the $p16^{INK4a}$-CDK4/6-RB senescence barrier

Normal melanocytes that acquire a mutation in *BRAF* do not readily progress to a melanoma. After a limited number of divisions, they enter a state of irreversible growth arrest known as senescence (Fig. 1). Benign nevi represent

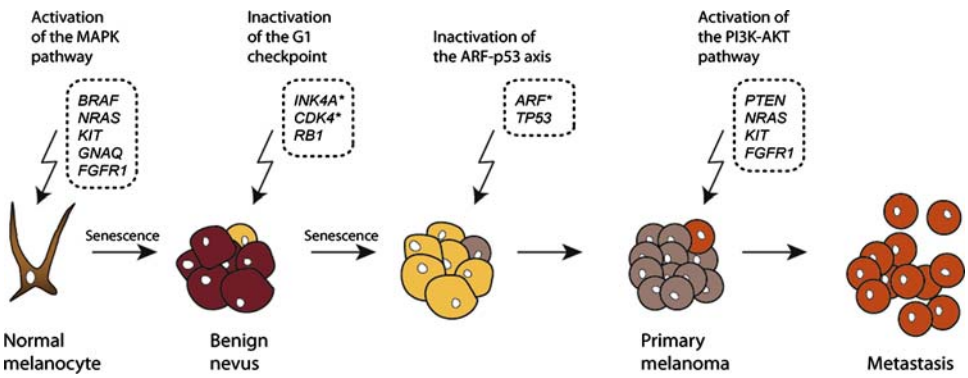

Fig. 1 A hypothetical model of melanoma development. Each step along the progression line is driven by the acquisition of changes in specific genes. Germline mutations in some of these genes have been found in melanoma kindreds (marked with an *asterisk*)

clusters of senescent cells that can remain quiescent for many years and will only rarely progress to melanoma. One of the most well-described senescence barriers in human cells involves $p16^{INK4a}$, which is encoded from the *CDKN2A* locus (Fig. 2). This locus also encodes the functionally and structurally unrelated tumor suppressor, $p14^{ARF}$ (Fig. 2). $p16^{INK4a}$ is produced from a transcript generated from exons 1α, 2 and 3, whereas $p14^{ARF}$ is produced, in an alternative reading frame (ARF), from a transcript comprising exons 1β, 2 and 3.

$p16^{INK4a}$ is an inhibitor of the cyclin-dependent kinases CDK4 and CDK6, and prevents S-phase entry by maintaining the retinoblastoma protein (RB) in an activated, hypophosphorylated state (Fig. 2). $p16^{INK4a}$ has been found to be inactivated in the vast majority of melanomas through mutation, deletion or promoter hypermethylation of *CDKN2A* (Bennett 2008a; Dahl and Guldberg 2007; de Snoo and Hayward 2005).

Genomic alterations affecting other components of the $p16^{INK4a}$-CDK4/6-RB senescence barrier have also been found in melanoma specimens, albeit at significantly lower frequencies than *CDKN2A* alterations. Somatic mutations in codons 22 and 24 of *CDK4* have been identified at frequencies up to 10% (Bennett 2008a; Dahl and Guldberg 2007; de Snoo and Hayward 2005). The mutant proteins do not bind $p16^{INK4a}$ and act as oncogenes as their RB kinase activity is no longer inhibited (Bartkova et al. 1996; Wölfel et al. 1995). Inactivating mutations on both alleles of *RB1* have been reported in three melanoma cell lines derived from the same patient (Bartkova et al. 1996), but no study has systematically examined the status of *RB1* in melanoma specimens. Mutations in *CDKN2A*, *CDK4* and *RB1* occur in a mutually exclusive manner (Bartkova et al. 1996; Walker et al. 1998), reflecting that they are all essential components of a functional unit involved in melanoma suppression.

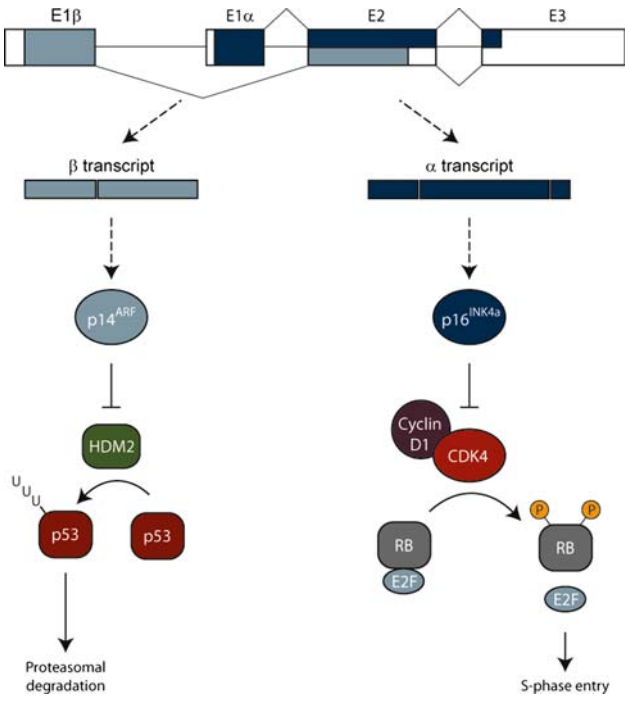

Fig. 2 The *CDKN2A* locus and its products. Exons 1α, 2 and 3 produces a mRNA that encodes $p16^{INK4A}$, whereas exons 1β, 2 and 3 encodes $p14^{ARF}$. The tumor-suppressor activity of $p16^{INK4A}$ has been ascribed to its ability to inhibit the cyclin-D-dependent kinases CDK4 and CDK6. Inactivation of $p16^{INK4A}$ leads to CDK-mediated phosphorylation of RB, which causes the release of E2F transcription factors, which are potent stimulators of cell-cycle entry. $p14^{ARF}$ activates the tumor suppressor p53 by inactivating HDM2, a ubiquitin ligase that targets p53 for degradation by the proteasome. Loss of $p14^{ARF}$ leads to HDM2-mediated inactivation of p53

Inactivation of the ARF-p53 senescence barrier

While inactivation of the $p16^{INK4a}$-CDK4/6-RB senescence barrier is a necessary step for melanocytes to bypass senescence, compelling evidence suggests that it is not sufficient. Notably, melanocytic cells that lack $p16^{INK4a}$ have an extended lifespan in culture compared with wildtype cells, but eventually undergo growth arrest characterized by senescence (Bennett 2003; Sviderskaya et al. 2003) (Fig. 1). This alternative senescence barrier in melanocytes is likely to involve p53, which is strongly up-regulated in $p16^{INK4a}$-deficient melanocytes that have reached senescence (Sviderskaya et al. 2003). The frequency of *TP53* mutations in melanoma-derived specimens is 5–25% (Bennett 2008a; Dahl and Guldberg 2007; de Snoo and Hayward 2005), which is relatively low compared with many other cancers, and may be explained by the frequent loss of $p14^{ARF}$ in melanoma through *CDKN2A* mutations and deletions. $p14^{ARF}$ is a positive regulator of p53 (Fig. 2), and loss of $p14^{ARF}$ function may have the same effect as loss of p53.

Activation of the PTEN-PI3K pathway

The tumor suppressor *PTEN* was first found to be deleted and mutated in melanoma in 1997 (Guldberg et al. 1997). Since this early study, *PTEN* mutations have been identified at overall frequencies of ~10% in uncultured melanomas and 30–50% in cell lines. PTEN is a negative regulator of the oncogenic phosphatidylinositol-3-kinase (PI3K) signaling pathway, which has fundamental importance for cell growth and survival (Cully et al. 2006), and inactivation of *PTEN* by deletion or mutation leads to constitutive activation of this pathway. *PTEN* mutations are found only rarely in primary melanomas (Birck et al. 2000; de Snoo and Hayward 2005; Guldberg et al. 1997), suggesting that activation of the PI3K pathway may be responsible for the late processes in melanoma development, i.e., invasion and metastasis.

Most melanoma metastases show hyperactive PI3K-AKT signaling, which can be ascribed to PTEN inactivation in <50% of the cases. In some of the remaining cases, oncogenic mutations in other genes have been found, which are also able to mediate constitutive activation of this pathway, including *NRAS* (Platz et al. 2007), *KIT* (Curtin et al. 2006a) and *PIK3CA* (encoding the catalytic subunit of PI3K) (Curtin et al. 2006b; Omholt et al. 2006). Several studies have shown that *NRAS* and *PTEN* mutations rarely occur in the same tumor and thus are likely to be functionally equivalent (Tsao et al. 2004b).

The genetic epidemiology of melanoma

Most melanomas occur as sporadic cases with no recognized familial component. However, the clustering of melanoma within families has been thoroughly documented in numerous epidemiological studies. Melanoma appears to be twice as common in persons with an affected parent, three times as common if a sibling is affected, and nine times as common if both a parent and a sibling are affected (Hemminki et al. 2003). Overall, up to 13% of patients with melanoma have at least one first-degree relative with the same cancer. This familial clustering most likely reflects both genetic and environmental factors. In families with multiple cases of melanoma, the pattern of susceptibility is usually consistent with autosomal dominant inheritance of a single gene. However, the majority of familial aggregations show a complex pattern of inheritance that cannot be explained solely by the transmission of a single gene. In most cases, therefore, melanoma is a complex trait influenced by genetic and environmental factors and their interactions.

The genetic melanoma predisposition factors identified to date can be stratified by risk profile into high-penetrance genes and low-penetrance alleles. While convenient for academic discussion, this distinction is arbitrary and does not reflect that the risk imposed by inherited genetic factors represents a broad continuum and there is likely to be some overlap between the two tiers. A number of human syndromes are associated with an elevated incidence of melanoma, including retinoblastoma (Fletcher et al. 2004), Werner syndrome (Goto et al. 1996), xeroderma pigmentosum (Kraemer et al. 1994) and neurofibromatosis (Guillot et al. 2004). These syndromes and the genes involved have been reviewed elsewhere (de Snoo and Hayward 2005) and will not be considered further here.

High-penetrance melanoma susceptibility genes

A major breakthrough in our understanding of hereditary melanoma occurred in the mid-1990s when it was shown that germline mutations in the *CDKN2A* locus and *CDK4* segregate in melanoma-prone kindreds in a dominant pattern (Hussussian et al. 1994; Kamb et al. 1994; Zuo et al. 1996). Since then, only little progress has been made in identifying genes responsible for familial melanoma, and *CDKN2A* and *CDK4* remain the only high-penetrance melanoma genes identified to date. It is not the intension here to describe the plethora of studies on these genes. Instead, we will extract the salient points from these studies and focus on recent larger multi-center studies and population-based risk studies.

CDKN2A

Virtually all persons who carry germline mutations in the *CDKN2A* locus are heterozygotes and thus carry a wildtype copy of the gene on the other chromosome. This copy may be lost by somatic mutation during melanoma progression, consistent with Knudson's two-hit hypothesis for tumor-suppressor inactivation. The most extensive study of the role of *CDKN2A* in familial melanoma was conducted by the Melanoma Genetics Consortium (GenoMEL; http://www.genomel.org), which comprises around 20 centers from Europe, Australia, North America and the Middle East. In one report from this Consortium (Goldstein et al. 2006), data on *CDKN2A* status was compiled on 466 high-risk families with three or more confirmed melanoma patients (a total of 2,137 melanoma patients). Overall, *CDKN2A* alterations (single-base changes, small insertions and deletions or, rarely, large deletions) were found in 40% of these families. There were 66 different mutations, of which 43 were unique and the remainder occurred in two or more families. In some geographic regions, single founder mutations accounted for the majority of mutant *CDKN2A* alleles, with the most extreme cases being Sweden, where one mutation was found in 11 out of 12 families, and the Netherlands where another mutation was found in 18 out of 20 families. The median age at melanoma diagnosis was significantly lower in carriers of *CDKN2A* mutations (36 years) than in patients from families with wildtype *CDKN2A* (45 years) (Goldstein et al. 2006).

Due to the unusual structure of *CDKN2A* (Fig. 2), mutations in this locus may affect $p16^{INK4a}$, $p14^{ARF}$, or both, depending on the localization and type of sequence alteration. The vast majority of the disease-associated mutations found in the GenoMEL study were predicted to affect $p16^{INK4a}$, and 40% of the mutations were in exon 1α, which is specific for $p16^{INK4a}$ (cf. Fig. 2). Furthermore, 8 out of 22 missense mutations in exon 2 had no predicted effect on $p14^{ARF}$, and no mutations in exon 2 affected $p14^{ARF}$ without affecting $p16^{INK4a}$. These data would suggest that there is selectivity for mutations affecting $p16^{INK4a}$. Nevertheless, in seven of the families, mutations or deletions in exon 1β,

which is $p14^{ARF}$-specific, were found to segregate with melanoma in an autosomal dominant pattern. Other researchers have also reported exon 1β-specific mutations in melanoma-prone kindreds (Hewitt et al. 2002; Randerson-Moor et al. 2001; Rizos et al. 2001). The pattern of germline *CDKN2A* mutations in melanoma families is similar to that of somatic *CDKN2A* mutations in melanoma samples (Forbes et al. 2008), with the majority of mutations affecting both $p14^{ARF}$ and $p16^{INK4a}$, some affecting $p16^{INK4a}$ only and a few affecting $p14^{ARF}$ only. Thus, although $p16^{INK4a}$ is targeted more often than $p14^{ARF}$, the spectra of inherited and somatic *CDKN2A* mutations are consistent with a role of both $p16^{INK4a}$ and $p14^{ARF}$ in melanoma suppression, consistent with their proposed role as independent mediators of melanocyte senescence (see Fig. 1).

One of the interesting observations in the GenoMEL study was a considerable variation in the frequency of *CDNK2A* mutations across the participating centers (Goldstein et al. 2007). Notably, this variation could not be ascribed to differences in the quality of mutation detection (Harland et al. 2008), but rather to differences in frequencies across the continents (Goldstein et al. 2007). The proportion of families with *CDNK2A* mutations was highest in Europe (57%), where the baseline incidence of melanoma is relatively low, and lowest in Australia (20%), where the melanoma incidence is high. Accordingly, in high-incidence areas, a significant proportion of familial clustering may be ascribed to high sun exposure and thus represent *CDNK2*-mutation phenocopies.

In families with *CDNK2A* mutations, penetrance was shown to be influenced by geographical location, with the highest penetrance observed in regions with the highest melanoma incidence (Bishop et al. 2002). Although this would suggest that melanoma development in *CDKN2A* mutation carriers is dependent on environmental exposures, it cannot be excluded that at least some of the geographic variation in penetrance can be explained by differences in mutational spectra. There is evidence suggesting that *CDKN2A* mutations affecting both $p16^{INK4a}$ and $p14^{ARF}$ are associated with a higher penetrance than mutations affecting $p16^{INK4a}$ alone (Berwick et al. 2006; Bishop et al. 2002). Thus, if different *CDKN2A* mutations confer different risks of melanoma, large interregional differences in penetrance would be expected as the frequencies of individual *CDKN2A* mutations vary significantly among populations.

One limitation of the GenoMEL study is that it is restricted to multiple-case families, which results in selection for families with higher risk of melanoma compared with population-based and unselected series of patients. Indeed, the GenoMEL study (Goldstein et al. 2007) as well as three other studies (Bishop et al. 2006; Eliason et al. 2006; Niendorf et al. 2006) have shown that the probability of finding a *CDKN2A* mutation increases with the number of recorded patients with melanoma in a family. Thus, estimates of the penetrance of *CDKN2A* mutations derived from multiple-case studies may not be representative of *CDKN2A* mutation carrier risks in the general population. In a large population-based study, the frequency of *CDKN2A* mutations was 1.2% in incident cases of first primary melanoma and 2.9% in patients with multiple primary melanomas (MPM) (Berwick et al. 2006). Interestingly, only 27% of the mutations found in the unselected series occurred in the reading frames common to $p16^{INK4a}$ and $p14^{ARF}$, whereas in the GenoMEL study of high-risk families, 64% of the families carried such mutations. This distribution of mutation types suggests that *CDKN2A* mutations are associated with a wide range of melanoma risks, and that a population-based approach will identify the lower-risk variants with greater frequency than studies of multiple-case families (Berwick et al. 2006).

Several studies have shown that carriers of *CDKN2A* mutations have an increased risk of cancers other than melanoma, in particular pancreatic cancer (Borg et al. 2000; Goldstein et al. 1995). In the GenoMEL study, 28% of families with a *CDKN2A* mutation had at least one case of pancreatic cancer, whereas this cancer was only observed in 6% of families with wildtype *CDKN2A* (Goldstein et al. 2006). There have been a number of reports suggesting that *CDKN2A* mutations that specifically affect $p14^{ARF}$ confer an increased risk of neural system tumors (Goldstein et al. 2006; Hewitt et al. 2002; Randerson-Moor et al. 2001; Rizos et al. 2001); however, this association needs to be confirmed in larger studies.

CDK4

Only two *CDK4* mutations have been found in the germline of melanoma patients, i.e., Arg24His and Arg24Cys. These mutations are also the most common somatic *CDK4* mutations found in melanoma (Bennett 2008a; Dahl and Guldberg 2007; de Snoo and Hayward 2005; Forbes et al. 2008) and have been shown to convert *CDK4* into an autosomal dominant oncogene. Other oncogene mutations frequently observed in melanoma specimens, such as those in *BRAF* and *NRAS*, are not found in the germline of melanoma-prone families (James et al. 2006). Germline *BRAF* mutations have been identified in the cardio-facio-cutaneous (CFC) syndrome; however, this syndrome is not associated with a higher risk of cancer (Niihori et al. 2006; Rodriguez-Viciana et al. 2006).

Germline mutations in *CDK4* are rare compared with *CDKN2A* mutations. Only 2% of the families in the GenoMEL carried *CDK4* mutations (Goldstein et al. 2006), and less than 15 such families have been reported worldwide (Helsing et al. 2008; Molven et al. 2005; Pjanova et al.

2009; Soufir et al. 1998, 2007; Zuo et al. 1996). In a Norwegian population-based study, three out of 390 patients diagnosed with MPM carried a *CDK4* mutation (0.8%), and these patients were all later confirmed to belong to a large melanoma-prone family (Molven et al. 2005). On the basis of the current data, *CDK4* mutations seem to be associated with the same pattern of inheritance and a similar median age at melanoma diagnosis as *CDKN2A* mutations. However, given the low frequency of *CDK4* mutations, very large population-based studies in diverse populations will be required to fully understand the role of this gene in melanoma susceptibility.

Low-penetrance melanoma susceptibility alleles

Numerous epidemiological studies have established that the risk of developing melanoma is directly related to pigmentation phenotype. Fair skin, poor tanning response, red or blonde hair and freckles are all known melanoma risk factors (Bliss et al. 1995). Our understanding of the molecular genetics of pigmentary traits has increased immensely over the past few years. Through systematic analysis of candidate genes and recent GWAS approaches, a large number of genetic determinants of skin, hair and eye color variation have been identified (Sturm 2009). Notably, a number of SNPs associated with genes involved in the melanin synthesis pathway have emerged as strong candidates for low-penetrance melanoma susceptibility factors (Table 1).

Melanin synthesis

Melanin is a complex mixture of tyrosine-derived biopolymers that is responsible for pigmentation of the skin, hair and eyes. In response to UVR, dermal melanocytes produce melanin in specialized intracellular organelles called melanosomes, which are subsequently distributed to the surrounding keratinocytes, where they accumulate on the sun-exposed side of the nuclei to shield the nuclear material from the damaging effects of UVR. Melanin is synthesized in two basic forms, the black photoprotective eumelanin and the reddish-yellow pheomelanin. The ratio of these two types of melanin determines the pigmentation phenotype of an individual. Pheomelanin is predominant in people with red hair, freckles and fair skin that tans poorly, the so-called red hair color (RHC) phenotype, whereas eumelanin is abundant in dark-complexioned individuals (Sturm 2009).

A key regulator of melanin synthesis is the melanocortin-1 receptor (MC1R), a seven-pass transmembrane G-protein-coupled receptor expressed on the cell surface of melanocytes. The binding of either of two melanotrophic keratinocyte-derived agonists, α-melanocyte stimulating

Table 1 Summary of low-penetrance melanoma susceptibility factors

Gene	Variant	*N* cases	*N* controls	Odds ratio (95% CI)	*P* value	References
MC1R	Val60Leu	1,903	3,162	1.15 (0.92–1.43)	0.01	Raimondi et al. (2008)
	(rs1805005 T)	1,947	11,173	1.13 (1.01–1.26)	0.04	Gudbjartsson et al. (2008)
	Asp84Glu	1,271	1,773	2.40 (1.50–3.84)	0.47	Raimondi et al. (2008)
	(rs1805006 A)	1,936	10,404	1.35 (0.94–1.96)	0.11	Gudbjartsson et al. (2008)
	Val92Met	1,635	2,631	1.22 (0.99–1.50)	0.31	Raimondi et al. (2008)
	(rs2228479 A)	1,965	10,536	1.03 (0.91–1.18)	0.63	Gudbjartsson et al. (2008)
	Arg142His	1,098	1,614	1.66 (1.01–2.75)	0.46	Raimondi et al. (2008)
	Arg151Cys	1,905	3,142	1.78 (1.45–2.20)	0.25	Raimondi et al. (2008)
	(rs1805007 T)	1,970	12,237	1.47 (1.28–1.70)	1.4×10^{-7}	Gudbjartsson et al. (2008)
	Ile155Thr	1,021	1,929	2.45 (1.32–4.55)	0.37	Raimondi et al. (2008)
	(rs1110400 C)	1,963	10,756	1.10 (0.78–1.55)	0.6	Gudbjartsson et al. (2008)
	Arg160Trp	1,900	3,164	1.43 (1.20–1.70)	0.64	Raimondi et al. (2008)
	(1805008 T)	1,950	11,837	1.26 (1.12–1.41)	1.1×10^{-4}	Gudbjartsson et al. (2008)
	Arg163Gln	1,617	2,730	1.42 (1.09–1.85)	0.36	Raimondi et al. (2008)
	(rs885479 A)	2,093	38,633	1.05 (0.89–1.22)	0.58	Gudbjartsson et al. (2008)
	Asp294His	1,657	2,816	1.77 (1.17–2.69)	0.08	Raimondi et al. (2008)
	(rs1805009 C)	1,961	10,699	1.68 (1.28–2.22)	2.2×10^{-4}	Gudbjartsson et al. (2008)
ASIP	rs1015362 G; rs4911414 T	2,111	40,094	1.45 (1.29–1.64)	1.2×10^{-9}	Gudbjartsson et al. (2008)
	rs910873; rs1885120	2,019	2,105	1.75 (1.53–2.01)	1.0×10^{-15}	Brown et al. (2008)
TYR	Arg402Gln (rs1126809 A)	2,111	40,599	1.21 (1.13–1.30)	2.8×10^{-7}	Gudbjartsson et al. (2008)
TYRP1	rs1408799 C	2,110	40,043	1.15 (1.06–1.24)	4.3×10^{-4}	Gudbjartsson et al. (2008)

hormone (α-MSH) and adrenocorticotropic hormone (ACTH), to this receptor initiates a signal cascade that acts through adenylate cyclase, leading to the production of cyclic adenosine monophosphate (cAMP) (Fig. 3). The elevation of intracellular cAMP levels leads to the up-regulation of microphthalmia transcription factor (MITF), which positively regulates genes involved in pigmentation, including tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1) and tyrosinase-related protein 2 (TYRP2). The effects of α-MSH and ACTH on the production of eumelanin is antagonized by agouti stimulating protein (ASIP) (Fig. 3).

MC1R

To date, nearly 60 nonsynonymous variants have been identified in *MC1R*, with the majority of allelic variation being restricted to European and Asian populations (Gerstenblith et al. 2007). Despite this high degree of polymorphism, genetic association studies have shown that some *MC1R* variants (denoted as "*R*" alleles) are more likely to contribute to the RHC phenotype than others ("*r*" alleles) (Duffy et al. 2004). In particular, the Arg151Cys, Arg160Trp and Asp294His variants of *MC1R* have been established as *R* alleles. Functional studies have shown that some MC1R variants elevate the cAMP production less efficiently than the wild-type receptor when stimulated with α-MSH (Beaumont et al. 2007), which can explain the reduced production of eumelanin in carriers of these variants.

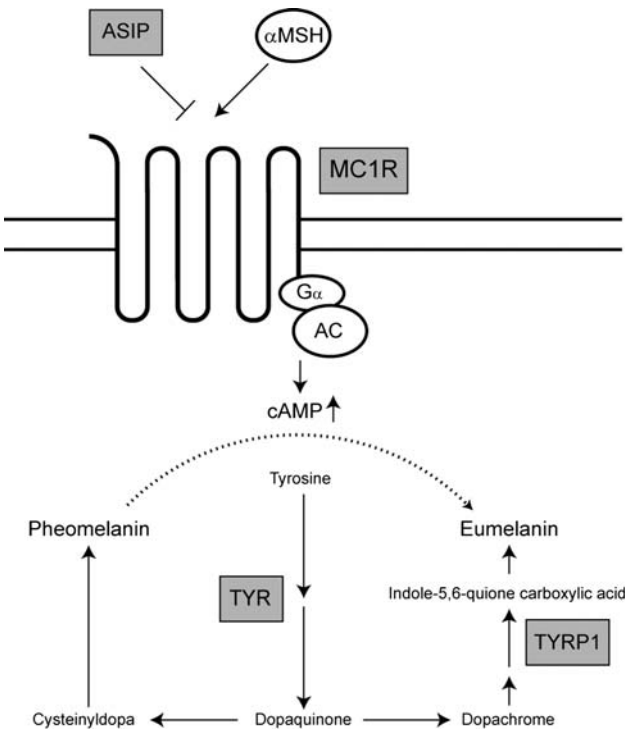

Fig. 3 Schematic outline of the biosynthesis of melanin highlighting genes that have been identified as potential low-penetrance melanoma susceptibility factors (*gray boxes*). *AC* adenylate cyclase, *Gα* α subunit of GTP-binding protein

As determinants of the RHC phenotype, variants of *MC1R* have long been suspected as melanoma susceptibility factors. In the first study that addressed this possibility, the *MC1R* genotype was determined in 43 unrelated melanoma patients and 44 controls. The frequency of *MC1R* variants was significantly higher in melanoma patients than in controls (47 vs. 18%), and the estimated risk of melanoma for individuals with one or two variant *MC1R* alleles was 3.9 relative to individuals who were homozygous for the consensus wildtype sequence (Valverde et al. 1996). Since this early contribution, numerous studies worldwide have reported similar results and thus identified *MC1R* as a low-penetrance melanoma gene. A meta-analysis (Raimondi et al. 2008) and a recent large-scale study (Gudbjartsson et al. 2008) have identified a total of nine *MC1R* variants that are associated with increased melanoma risk (Table 1). Interestingly, this risk persists after adjustment for hair color and skin type, suggesting that some *MC1R* alleles may increase the risk of melanoma beyond their effect on pigmentation (Kennedy et al. 2001; Landi et al. 2005; Palmer et al. 2000).

The risk of melanoma is related to the allelic status of *MC1R*. Two studies have shown that individuals who are homozygous or compound heterozygous for specific *MC1R* variants (e.g., the *R* alleles) have a four to fivefold higher risk of developing melanoma than individuals with two wildtype alleles, and that the risk is two to threefold higher in individuals who carry a single variant allele (Kennedy et al. 2001; Palmer et al. 2000). The higher risk in heterozygotes suggests that at least some *MC1R* variants may exert a dominant-negative effect. This notion has been corroborated by in vitro expression studies, showing that MC1R is able to undergo constitutive homo- and heterodimerization early in receptor ontogeny (Mandrika et al. 2005; Sanchez-Laorden et al. 2006), and that variant MC1R can have a negative effect on the expression of the wild-type receptor on the cell surface. It has been suggested that heterodimerization between wild-type and variant MC1R leads to intracellular retention of the receptor complex (Beaumont et al. 2007), which would explain why the variants correlate with a decreased ability to elevate intracellular cAMP levels and the existence of a heterozygote effect of *MC1R* variants on melanoma risk.

ASIP, *TYR* and *TYRP1*

The association between pigmentary traits and melanoma susceptibility was assessed in a recent large-scale study conducted in populations of European ancestry, comprising 2,121 individuals with melanoma and more than 40,000

controls (Gudbjartsson et al. 2008). In addition to the known *MC1R* variants, the authors studied 11 sequence variants at eight loci, which had previously been identified as determinants of hair, eye and skin pigmentation through GWAS approaches (Sulem et al. 2007, 2008). Variants at three of these eight loci showed significant association with melanoma. However, it is important to emphasize that a significant genome-wide association signal does not necessarily implicate a particular SNP as a causal variant for melanoma risk. Indeed, many SNPs associated with pigmentary traits map to regions without any known protein-coding genes and, therefore, provide no information about the causal functional DNA variants other than their possible chromosomal localization (Sulem et al. 2007, 2008).

The most significant association was found for an extended haplotype near *ASIP* on chromosome 20q11.22 with an odds ratio (OR) of 1.45 (Gudbjartsson et al. 2008). This gene is a promising melanoma susceptibility candidate as ASIP antagonizes the binding of α-MSH to MC1R and stimulates the production of pheomelanin (Fig. 3). In theory, gain-of-function mutations in *ASIP* could phenocopy the effects of the loss-of-function variants in *MC1R* and thus be associated with an increased risk of melanoma (Bastian and Pinkel 2008). To date, no variants have been identified in the coding regions of *ASIP*, and a SNP in the 3′ untranslated region of the gene is not associated with an increased risk of melanoma (Landi et al. 2005). An independent GWAS comprising about 2,019 melanoma patients and 2,105 controls identified two other sequence variants on 20q11.22, which were associated with melanoma risk (OR = 1.8). However, fine mapping of this region did not find an association with *ASIP* but rather with a more telomeric region that includes several other candidate loci (Brown et al. 2008). Thus, the possible role of *ASIP* in melanoma susceptibility remains unresolved.

The other variants found to be associated with an increased risk for melanoma include a SNP located near the *TYRP1* locus (OR = 1.15) and a coding sequence variant in *TYR* (p.R402Q; OR = 1.21) (Gudbjartsson et al. 2008). The *TYR* variant affects eye color and tanning response and encodes an enzyme that is unable to exit the endoplasmic reticulum at physiologic temperatures (Berson et al. 2000). This variant is also associated with a temperature-sensitive form of oculocutaneous albinism with peripheral pigmentation (King et al. 1991).

Gene–gene and gene–environment interactions

The development of melanoma is a sequential and selective process that contains many stochastic elements (cf. Fig. 1). Each step along the progression line requires spontaneous mutations in specific genes, and the entire sequence of events may or may not occur during the lifetime of an individual. Inherited gene variants that increase the risk of melanoma do not seem to alter the genetic requirements of the tumorigenic process, but rather increase the likelihood that they will occur.

The mechanisms by which high-penetrance genes and low-penetrance alleles contribute to melanoma predisposition are probably not the same. The two known high-penetrance genes, *CDKN2A* and *CDK4*, encode key mediators of melanocyte senescence (Fig. 2) (Bennett 2003), and mutations in these genes seem to be required for tumor progression (Fig. 1). If *CDKN2A* or *CDK4* is mutated in the germline, the number of spontaneous genetic hits required for tumor formation is reduced by one, which will increase the probability that the entire sequence of events will occur. Thus, carriers of *CDKN2A* and *CDK4* mutations will, on average, develop melanoma earlier in life than individuals with normal copies of these genes. Due to the stochastic nature of the tumorigenic process, some *CDKN2A* and *CDK4* mutation carriers will not develop melanoma, which may explain in part the incomplete penetrance of these mutations. Incomplete penetrance may also be a result of differences in sun exposure and co-inheritance of modifier genes (discussed below).

The contribution of low-penetrance alleles (e.g., *MC1R*, *ASIP*, *TYR* and *TYRP1* variants) to melanoma susceptibility is less clear. These variants arose during human evolution, possibly as a result of positive selection for reduced pigmentation in non-sunny climates. As UVR is a known mutagenic carcinogen (Pfeifer et al. 2005), an obvious explanation for the increased incidence of melanoma among fair-skinned people would be an increased rate of UVR-induced mutations in melanocytic cells as a result of reduced constitutive pigmentation. However, the majority of mutations found in melanocytic tumors are not typical UV-signature mutations (i.e., single C-to-T or tandem CC-to-TT transition mutations), in contrast to non-melanoma skin cancer, where these mutation types predominate (Hocker and Tsao 2007). Further, as the V600E *BRAF* mutation is also found at appreciable frequencies in internal cancers, such as cancers arising in the colon, thyroid and ovary (Davies et al. 2002; Kimura et al. 2003), it is still a matter of controversy whether the direct mutagenic effect of UVR is a major contributor to melanoma development. Several studies have suggested an indirect effect of UVR, which is related to pigmentation type (Bennett 2008b). For example, it has been shown that pheomelanin is photoreactive and capable of producing reactive oxygen species, which could contribute to melanoma development by inducing various types of mutations (Brenner and Hearing 2008; Wenczl et al. 1998).

Recently, Bastian and coworkers demonstrated an interesting link between inherited low-penetrance alleles and

spontaneous oncogenic mutations in melanoma (Landi et al. 2006). The rate of *BRAF* mutations was found to be significantly higher in melanomas from individuals who carried *MC1R* variants than in homozygotes for the wild-type *MC1R* allele. This association suggests that *BRAF* mutations arise more readily in a MC1R-deficient background, or, alternatively, that melanocytic cells with *BRAF* mutations are more viable when MC1R signaling is impaired (Dhomen and Marais 2007). It will be interesting to learn about other possible associations between inherited risk factors and specific somatic mutations in melanoma development.

While gene–environment interactions have been shown to play an important role in melanoma susceptibility, clarity is more limited when it comes to interactions among the various types of genetic risk factors. The best documented example of a gene–gene interaction comes from two studies of *MC1R* variants in *CDKN2A*-mutation carriers living in Australia and the Netherlands, respectively (Box et al. 2001; van der Velden et al. 2001). In the Australian cohort, co-inheritance of an *MC1R* variant (Arg151Cys, Arg160Trp or Asp294His) increased the penetrance of *CDKN2A* mutations from 50 to 84% and decreased the mean age of onset of melanoma with approximately 20 years (Box et al. 2001). In the Dutch cohort, the penetrance of one of the known founder *CDKN2A* mutations ("p16-Leiden") was increased from 18% in individuals with wildtype *MC1R* to 35% in carriers of one *MC1R* variant and 55% in carriers of two *MC1R* variants. In these carriers, however, there was no observed effect of *MC1R* status on the mean age of onset of melanoma (van der Velden et al. 2001). This disparity between the two cohorts may in part reflect differences in UVR exposure.

The variants in *MC1R*, *ASIP*, *TYR* and *TYRP1* have been identified as independent low-penetrance susceptibility factors, but little is known about the combined risk of two or more factors. As these variants all act in the melanin synthesis pathway (Fig. 3), they may not necessarily act independently and multiplicatively. Very large population-based studies will be required to investigate the combined effect of these variants in conferring melanoma susceptibility and will be hampered by the small effect sizes (OR down to 1.15) and the large number of possible allele combinations.

Concluding remarks

Since 1857 when the English general practitioner William Norris first proposed that melanoma can be a hereditary disease (Norris 1857), our understanding of genetic predisposition to melanoma has advanced significantly. However, we are still far from the full picture. The identification of *CDKN2A* and *CDK4* mutations as the inherited component in some high-risk families was a major achievement, but these genes account for less than 30% of familial cases of melanoma and their overall contribution to disease burden is low. One of the goals of future research will be to identify additional high-penetrance dominant predisposition loci. One such locus has been mapped to chromosome 1p22 by linkage analysis (Gillanders et al. 2003), and another locus was mapped to 9q21 in three families with multiple cases of ocular and cutaneous melanoma (Jönsson et al. 2005). However, sequencing of candidate genes in these regions have so far been unsuccessful. Another important goal will be to identify the causative variants responsible for the association of certain SNPs with melanoma susceptibility, including the variants near *ASIP* and *TYRP1* (Table 1).

Although a combination of genome-wide linkage analyses, resequencing and other technologies may assist in identifying the remaining genetic factors of melanoma susceptibility, considerable challenges lie ahead. As for most other complex traits, the spectrum of genetic factors contributing to melanoma risk is likely to include a small number of variants with a large effect on melanoma risk and a large number of variants each having a small effect. While some of the variants responsible for the extremes of this spectrum have already been identified (i.e., rare high-penetrance factors such as *CDKN2A* and *CDK4* and common low-penetrance factors such as *MC1R*, *ASIP*, *TYR* and *TYRP1*), it will be more technically demanding to identify factors in the middle part of the spectrum. In some familial clustering of melanoma, an identifiable genetic component may not even exist. As the incidence of melanoma in the general population increases, the number of families in which more members are affected by chance will also increase, which will dilute the contribution from genetic factors. It will require a multidisciplinary approach involving a wide range of expertise to register, qualify and validate all the emerging components of melanoma risk.

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