Genetic Determinants of Cutaneous Melanoma Predisposition

Durga Udayakumar, PhD* Bisundev Mahato,[†] Michele Gabree, MGC[‡] and Hensin Tsao, MD, PhD[*,†,‡]

In the last 2 decades, advances in genomic technologies and molecular biology have accelerated the identification of multiple genetic loci that confer risk for cutaneous melanoma. The risk alleles range from rarely occurring, high-risk variants with a strong familial predisposition to low-risk to moderate-risk variants with modest melanoma association. Although the high-risk alleles are limited to the *CDKN2A* and *CDK4* loci, the authors of recent genome-wide association studies have uncovered a set of variants in pigmentation loci that contribute to low risk. A biological validation of these new findings would provide greater understanding of the disease. In this review we describe some of the important risk loci and their association to risk of developing cutaneous melanoma and also address the current clinical challenges in *CDKN2A* genetic testing.
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During the last 3 decades, the incidence of cutaneous melanoma (CM) has increased largely in Caucasian populations around the world.[1-3] In the United States, the estimated number of new cases of invasive melanoma in 2010 is 62,480, of which an annual estimated mortality rate is at 2.3% for all races and 2.8% in white populations alone.[1] The outcome for melanoma is highly dependent on the stage of the disease.[4] On one end of the spectrum is the primary CM, which when excised appropriately, is highly curable. However, by contrast, metastatic melanoma comes with a very poor prognosis despite treatment,[5-6] and patients with stage IV melanoma have an expected 9% to 15% rate of survival[4] at 5 years. Because early disease is significantly more favorable in prognosis, there has been much focus on the need to identify those at greatest risk for melanoma. Recent advances in technology and molecular biology have enhanced our ability to more accurately personalize melanoma risk assessment.[7,8]

Cutaneous melanoma is a cancer in which a combination of genetic and environmental factors clearly influences the onset of disease.[9] High-risk alleles often are expressed as familial clusters, whereas lower-risk alleles result in sporadic cases.[10-12] The purpose of this review is to discuss current knowledge about the individual risk loci that modulate risk for melanoma. Also, we have addressed some of the current progress and limitations of genetic testing and the strategies for establishing risk intervention programs to promote effective risk management recommendations.

High-Risk Alleles in Melanoma Predisposition

Hereditary melanoma typically arises in an autosomal-dominant pattern within multiplex families. Often, there is an association with clinically atypical nevi, although patients may harbor nevi without melanoma and vice versa.[13,14] Progress in genetic methodology in the late 1980s and early 1990s led to the identification of germ line mutations in p16Ink4a (now *CDKN2A*), which is located on chromosome 9p21.[15,16] Shortly thereafter, germline mutations of *CDK4* were also recognized in certain kindreds that lacked heritable *CDKN2A* alterations.[17,18] Thus, within a few short years in the mid-1990s, the molecular basis of hereditary melanoma was quickly established in a subset of melanoma-prone kindreds. These loci account for approximately 20% to 57% of disease susceptibility,[19] thereby suggesting that other high-risk loci still exist. At the tail end of the successful completion of the Human Genome Project, there has been intense interest in enumerating lower-risk disease alleles

*Department of Dermatology, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA.
†Harvard Medical School, Boston, MA.
‡Center for Cancer Risk Assessment, Massachusetts General Hospital, Boston, MA.
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Address reprint requests to Hensin Tsao, MD, PhD, Department of Dermatology and Melanoma Genetics Program, Bartlett 622, 48 Blossom St, Boston, MA 02114. E-mail: htsao@partners.org
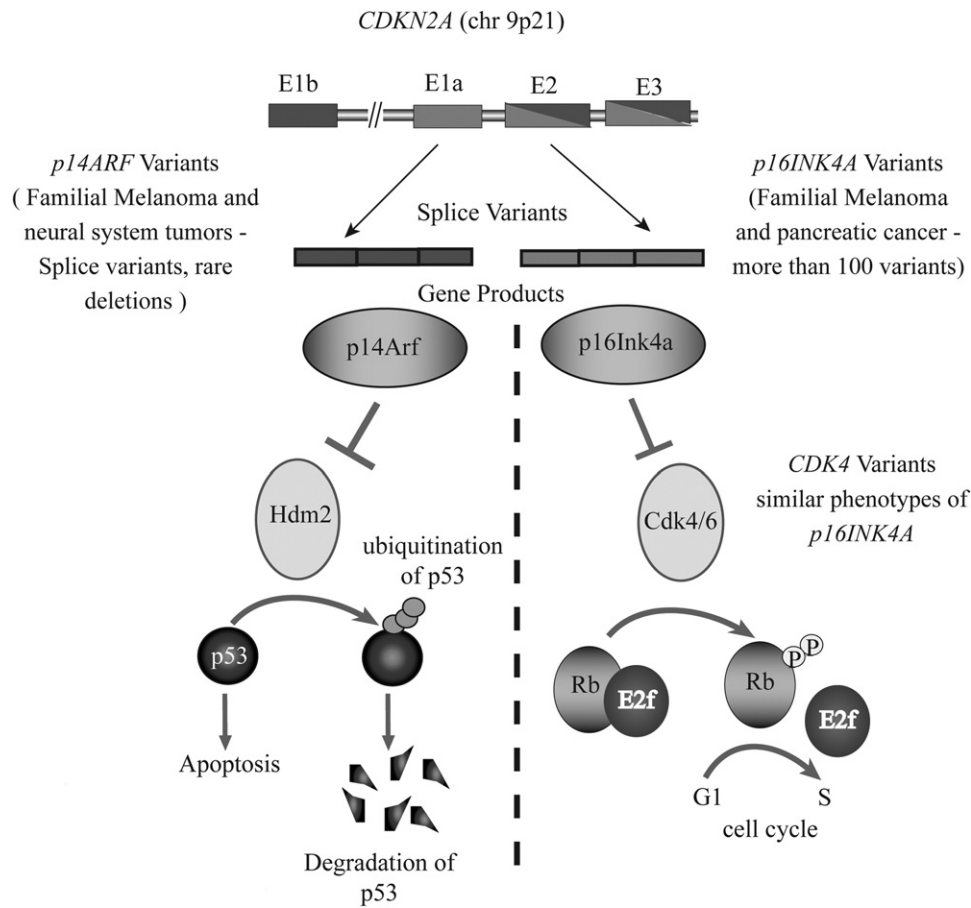
Figure 1 High-risk melanoma predisposition loci. The schematic represents the signaling pathways controlled by *CDKN2A* loci. The 2 different protein products encoded by the splice variants - p14Arf and p16Ink4a, converge on the same cellular function—cell-cycle regulation. The p14Arf regulates the p53 tumor suppressor network and the P16Ink4a regulates the Rb network. Variants in p14Arf, p16Ink4a, Cdk4, and Rb contribute to deregulated signaling and uncontrolled cell proliferation.

through genome-wide association studies (GWAS). As outlined in this article, the combination of familial and population genetics have led to a much larger and richer view of melanoma predisposition.

CDKN2A

The *CDKN2A* locus constitutes 4 exons that encode 2 functionally distinct proteins through alternate splicing events, leading to 2 very potent tumor suppressors: p16Ink4a and p14Arf (Fig. 1).[15,16] Tumor-derived alleles of p16Ink4a result in mutant proteins that are no longer able to inhibit cyclin-dependent protein kinases (Cdks)-4 and -6 and are thus no longer able to suppress the phosphorylation of the retinoblastoma protein.[20,21] When the Rb protein becomes hyperphosphorylated, the cell transitions from G1 to the S-phase and begins to proliferate. p14Arf binds to Hdm2 and promotes rapid degradation of Hdm2, thus stabilizing p53[22-25]; thus, in the context of tumors, the loss of p14Arf results in an indirect loss of p53 function and deregulation of cell cycling and DNA damage signaling.[26] A vast array of *CDKN2A* mutations have been identified in families throughout the world, and most occur in exons 1-alpha and 2.[17,27,28] Interestingly, the large number of mutations in exon

1-alpha suggests that p16Ink4a and not p14Arf[18] may be preferentially targeted. However, mutations in the p14Arf-specific exon 1-beta locus have been described, thereby indicating that p14Arf is a bona-fide melanoma risk gene.[29-31] In a recent study, the rate of germline p14Arf mutations without coinheritance of p16Ink4a and Cdk4 mutations was estimated to be 3.2%,[32] which is consistent with a previous multicenter study.[33] Two of the reported families shared the g.193 + 1A > G splice-site mutation, and one family had a novel missense mutation g.161G > A, which causes the substitution of the arginine in position 54 with a histidine. A *CDKN2A* locus-specific database that lists all common germline variants is publicly available (https://biodesktop.uvm.edu/perl/p16).

Investigators in several studies have attempted to estimate *CDKN2A* mutation penetrance, defined as the observed risk of developing melanoma over time for a mutation carrier. A large GenoMEL (The International Melanoma Genetics Consortium) study, whose authors analyzed 80 families from 3 continents, estimated *CDKN2A* mutation penetrance to be 0.30 (95% confidence interval (CI) = 0.12-0.62) by age 50 and 0.67 (95% CI = 0.31-0.96) by age 80. There was signif-

icant variation in the mutation rate on the basis of population geography ($P = 0.003$). By age 50 years, *CDKN2A* mutation penetrance reached 0.13 in Europe, 0.50 in the United States, and 0.32 in Australia; by age 80 years it was 0.58 in Europe, 0.76 in the United States, and 0.91 in Australia. The variation as the result of geographic location suggests that other factors, such as degree of sun exposure or other coinherited gene modifiers, also contribute to the overall risk.

Unbiased estimates of *CDKN2A* penetrance have also been performed on the general CM population. The relative risk of a subsequent melanoma among patients with the mutations in *CDKN2A* with previously diagnosed primary melanoma is estimated to be 4.3 (95% CI = 2.3-7.7).[34,35] Population-based ascertainment leads to lower penetrance estimates (0.14 [95% CI = 8%-22%] by age 50, 0.24 [95% CI =15%-34%] by age 70, and 0.28 [95% CI =18%-40%] by age 80) compared with familial-based ascertainment, most likely because of shared genetic modifiers and exposures within families.[27] Some of the earlier studies have also shown that *CDKN2A* mutations are predominantly found in high-risk families (9 of 87%, 10.3%), thus, the contribution of such a mutation to the overall burden of CM is only 0.2%.[2,36] The melanoma penetrance is also greater (1.2%) in familial clusters with multiple primary melanoma (MPM) when compared with those with single primary melanoma.[27] The occurrence of *CDKN2A* mutations in patients with MPM regardless of familial history, albeit less frequent (8%-15%), could be a significant predictor of genetic susceptibility to melanoma during melanoma risk assessments.

Mutations in *CDKN2A* have also been associated with an increased risk of pancreatic cancer.[37] In the GenoMEL analysis of 385 families, 72% of families with 1 reported patient with pancreatic cancer had mutations (31/43) and 81% of families with ≥2 patients with pancreatic cancer had mutations (13/16).[19] The role of screening in the prevention of pancreatic cancer is still unclear, and thus, the impact of *CDKN2A* testing on pancreatic cancer management has not yet established.

CDK4

Cyclin-dependent kinase 4 (Cdk4), which physically and genetically interacts with the p16Ink4a (Fig. 1), is another important high-risk locus that that been implicated in melanoma risk. A small number of melanoma-prone families harbor mutations in *CDK4*. Both somatic and germline mutations of *CDK4* have been identified in melanoma cell lines and in families,[18,38] respectively. Recurrent mutations at codon 24 (Arg24His and Arg24 Cys) result in an autosomal-dominant oncogenic form of Cdk4, which is resistant to p16Ink4a inhibition.[17,18,39,40] Because the 2 proteins directly interact, the consequences of p16Ink4a loss and Cdk4 activation are similar; thus, carriers of *CDKN2A* and *CDK4* mutations also share a similar phenotype.[41] The Rb protein, which is downstream of p16Ink4a and Cdk4, is an essential gatekeeper for hereditary retinoblastoma. In patients cured of bilateral retinoblastoma (presumed *RB1* mutation carriers), the estimates of increased lifetime risk of melanoma range from 4-fold to 80-fold (reviewed in Chin et al[42]). Hence,

mutations in the entire *CDKN2A/CDK4/Rb* pathway appear to play a significant role in melanoma pathogenesis.

Low- to Moderate-Risk Susceptibility Alleles

With the Human Genome Project, it quickly became clear that single nucleotide changes, or polymorphisms (SNPs), occur throughout the human genome. These millions of variants have been recently leveraged in a host of GWAS. During the past few years, many GWAS analyses have led to the discovery of chromosomal regions linked to more common diseases and traits, including melanoma, pigmentation and nevus density. These GWAS have propelled the field forward by identifying many low to moderate risk loci that underlie sporadic CM formation (reviewed by Udayakumar and Tsao[43]).

MC1R

There are more than 120 genes that influence pigmentation. Recent comprehensive meta-analyses have directly linked pigmentation traits with the risk of developing melanoma.[44,45] A key pigmentation gene, melanocortin-1-receptor (*MC1R*), has been recently implicated as a low to moderate melanoma predisposition gene (reviewed in[43,46-49]). It is a 7-transmembrane protein that belongs to the family of G protein-coupled receptors (Fig. 2). When alpha-melanocyte stimulating hormone (alpha-MSH) binds to its receptor, Mc1r, it activates a G protein, which in turn stimulates the synthesis of cAMP through adenylate cyclase (AC). The microphthalmia transcription factor (Mitf) is subsequently induced, which leads to up-regulation of the pigment synthetic genes and eumelanin synthesis. Mutations in *MC1R* abrogate this pathway; therefore, melanocytes are enriched for pheomelanin, and patients exhibit increased freckling, red hair color (RHC), and enhanced photosensitivity.[50,51] A systematic study conducted by Raimondi and colleagues[51] investigated all *MC1R* variants and classified them based on degree of association with the RHC phenotype:

a. Strong association with more frequency: 4 "R" *MC1R* variants (p.Asp84Glu, p.Arg151Cys, p.Arg160Trp, and p.Asp294His)
b. Familial association with fewer frequency: 2 "R" alleles (p.Arg142His and p.Ile155Thr)
c. Weak Association: 3 "r" alleles (p.Val60Leu, p.Val92Met, and p.Arg163Gln)

Except for p.Val60Leu and p.Val92Met, both of which showed lack of significant association to melanoma risk, all other variants appear significantly associated with melanoma risk, with odds ratios ranging from 1.42 for p.Arg163Gln to 2.45 for p.Ile155Thr. Some *MC1R* variants that correlate well with skin/hair color and poor tanning ability (80% in patients with RHC and/or fair skin, 20% in patients with brown or black hair) show up to a 3.9-fold increase in CM risk.[52] Interestingly, the authors of several other studies have uncovered risk variants that show more complex genetic interactions. Some *MC1R* variants conferred little or no increased

risk of CM among one cohort versus another cohort from different geographic location.[53] Some variants conferred melanoma risk and no association with pigmentation phenotypes.[54] This complexity may reflect significant environmental inputs that have not been fully annotated in all studies. The presence of multiple *MC1R* variants has been significantly associated with melanoma especially among individuals with Multiple Primary Melanomas (MPMs). In addition, an inverse relationship has been observed between the number of *MC1R* variants with decreased median age at diagnosis.[55] Lastly, *MC1R* variants can also modify *CDKN2A* penetrance. In one study, the presence of *MC1R* variants Arg151Cys, Arg160Trp and Asp294His, in addition to *CDKN2A* mutation, increased the penetrance of *CDKN2A* mutations from 50% to 84% with a mean onset age of 37.8 years.[56]

Other Low-Risk Alleles

In recent years, several groups have isolated many novel variants associated with hair and eye pigmentation, skin sensitivity to sun, and freckling.[53,57-59] Some of the significant loci identified by several studies include *SLC24A4, KITLG, 6p25.3, TYR, OCA2, TPCN2, ASIP,* and *TYRP1*.[43] The *ASIP* locus ($P = 1.2 \times 10^{-9}$; OR = 1.45), coding variant in *TYR* ($P = 2.8 \times 10^{-7}$; combined or = 1.21) and a noncoding SNP at the tyrosinase-related protein 1 (*TYRP1*) locus are sig-
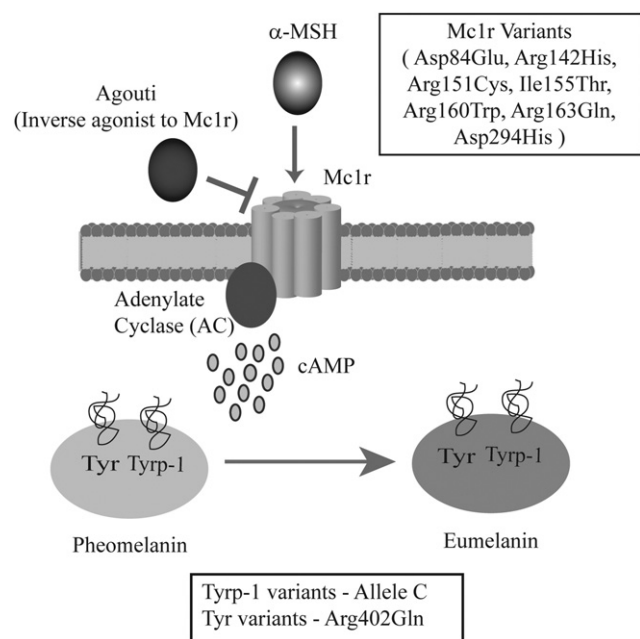
Figure 2 Mc1r pigmentation pathway implicated in melanoma risk. Alpha-melanocyte stimulating hormone (α-MSH) serves as a ligand of Mc1r. Agouti protein antagonizes this interaction. Binding of MSH to Mc1r stimulates adenylate cyclase to generate cAMP. cAMP is one of the key mediators of a series of events that leads to the conversion of pheomelanin to a photoprotective eumelanin during melanogenesis. The tyrosinase (Tyr) and the tyrosine-related protein kinase 1 (Tyrp-1) are the key enzymes that regulate this process. The variants of the *MC1R, TYR,* and *TYRP-1,* presumably lead to pigmentation differences, photosensitivity, and predisposition to cutaneous melanoma.

nificantly associated with melanoma risk.[58] Larger population-based GWAS studies identified other SNPs that show significant association with melanoma risk.[54,60-62] Others have reported low-risk variants, including methylthioadenosine phosphorylase (*MTAP*) gene that flanks the *CDKN2A* region, polymorphisms in the EGF, glutathione *S*-transferase (*GSTM1*), cytochrome P450 debrisoquine hydroxylase (*CYP2D6*), and vitamin D receptor gene (*VDR*) locus.[63-66] Further studies are needed to biologically validate these loci in the pathogenesis of melanoma.

Genetic Testing and Risk Management

Genetic testing for *CDKN2A* mutations is available clinically and on a research basis. Currently, the Melanoma Genetics Consortium and the American Society of Clinical Oncologists caution against the routine use of clinical *CDNK2A* testing for patients with hereditary melanoma because of the difficulty in interpreting test results, the potentially limited impact of the result on clinical management, and concerns for genetic discrimination.[36,67,68] Although, as discussed previously, mutations in this gene are associated with a significant risk for melanoma, it has also been noted that both families who test negative for a *CDKN2A* gene mutation as well as patients who test negative for a familial *CDKN2A* gene mutation remain at a risk greater than that of the general population to develop melanoma.[10,69]

The following features have been associated with a moderate to high risk for a hereditary melanoma predisposition syndrome: (1) multiple primary melanomas[27,70,71]; (2) family history of melanoma[71-73]; and/or (3) the presence of melanoma in addition to another related cancer, such as pancreatic cancer, on the same side of the family.[33,74-76] Early age of melanoma onset in patients with a negative family history has not been shown to increase the risk for a *CDKN2A* mutation.[27,71,73] Recently, MelaPRO, a risk-assessment model, was developed to assess the likelihood of a *CDKN2A* mutation in a person on the basis of personal and family history, as well as geographic penetrance. MelaPRO and other such models aid in the identification of patients and families at significant risk for melanoma who may benefit from further discussion regarding the option of genetic testing.[77]

Patients considering genetic testing for *CDKN2A* gene mutations should be informed of the possible test results, including positive, negative, and variant of uncertain significance, and the potential implications the test result could have on medical management. Informed consent for *CDKN2A* testing should also include a discussion regarding the cost of testing, genetic discrimination, and the possible psychological impact of testing.[68] In addition, it is important that individuals who opt for *CKDN2A* testing are aware of the increased risk for pancreatic cancer in some families, as well as the current lack of effective screening guidelines for pancreatic cancer.[37,74,75]

Conclusions

In this review we have discussed some of the very important loci reported to be involved in melanoma susceptibility and discussed other loci that have been implicated in the melanoma susceptibility process either as an independent risk factor or as a modifier locus. Although the identification of these low-risk alleles has stimulated much enthusiasm in the field, an understanding of the biological consequences and clinical utility of these findings remains to be established.

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