



Genetics in Melanoma

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Abstract

Melanoma is the leading cause of death from skin tumors worldwide, with an annual increase in incidence over the past decade. The molecular mechanisms involved in melanoma pathogenesis are beginning to be unraveled. While a family history of melanoma and exposure to ultraviolet irradiation have been known for years as risk factors in melanoma development, the precise genes involved in inherited predisposition were defined only in the past decade. Germline mutations in two genes that play a pivotal role in controlling cell cycle and division – *CDKN2A* and cyclin-dependent kinase 4 (*CDK4*) – have been detected in autosomal, dominant, high penetrance familial melanoma cases. In addition to these two highly penetrant genes, germline mutations and polymorphisms in a few low penetrance genes have been reported in familial melanoma cases: melanocortin-1 receptor, epidermal growth factor, glutathione s-transferase M1, cytochrome p450 debrisoquine hydroxylase locus (*CYP2D6*) and vitamin D receptor.

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Melanoma is diagnosed worldwide, accounting for about 3% of all malignancies, and is the leading cause of death from skin tumors. It is the most frequent tumor in men 20–40 years old, and the second most frequent tumor after breast cancer in women aged 20–40 [1]. Melanoma incidence varies by latitude and altitude worldwide, with areas closer to the equator and higher in altitude having the highest rates [2]. Melanoma rates also vary by skin pigmentation and sun exposure patterns in any given population. In Israel, the incidence has risen sevenfold over the past 20 years, whereas it doubled in the United States over the past 35 years; the worldwide incidence continues to rise by 5% annually [3]. Several risk factors have been identified as contributors to melanoma development. These include a family history of melanoma, skin complex, and number and type of skin nevi.

Family history

A family history of melanoma is one of the most significant risk factors for developing the neoplasm. A "positive family history of cancer relevant to melanoma" is defined as having two or more first-degree relatives with melanoma [4]. Phenotypically, histologically

and clinically, familial melanoma is indistinguishable from sporadic cases in most parameters. However, earlier age at diagnosis and multiple primary melanomas are more frequent than in sporadic cases [5]. A positive family history can be elicited in 0.6–12.5% of incidental melanoma cases, with higher percentages noted in geographic regions with a high melanoma occurrence rate. First-degree relatives of individuals with newly diagnosed melanoma have a twofold increase in the risk to develop melanoma [4].

Susceptibility genes: high penetrance genes

Germline mutations in two genes confer susceptibility to melanoma within high risk families – *CDKN2A* and cyclin-dependent kinase 4 (*CDK4*). Although, mutations in *CDKN2A* and *CDK4* account for only a small percentage of familial melanoma cases, both have high

*Two major groups of genes are involved
in malignant melanoma predisposition:
high penetrance and low penetrance genes*

penetrance (*CDKN2A*: 0.3-0.67, *CDK4*: 0.63). Both genes are involved in controlling cell cycle and cell division; *CDKN2A* is a tumor suppressor gene and *CDK4* is an oncogene. *CDKN2A* gene encodes for two proteins, p16 and p14ARF, both transcribed from different first exons but utilizing the same second and third exons. The principal action of p16 is to regulate G1 phase exit by inhibiting *CDK4*-mediated phosphorylation of the retinoblastoma protein. p14ARF acts via the p53 pathway to induce cell cycle arrest or apoptosis in response to hyperproliferative oncogenic signals [4].

CDKN2A

CDKN2A, the first high penetrance melanoma susceptibility gene, is located on chromosome 9p21. Germline mutations were initially reported in this gene in 1995 [6]. Since then, genetic analyses in ethnically diverse populations have reported a variety of germline mutations in melanoma-prone families, in melanoma cases diagnosed at a young age, or in multiple primary tumors [7].

Melanoma-associated mutations occur throughout the *CDKN2A* locus, as predicted by the fact that this is a tumor suppressor gene and they are missense mutations, thus making mutation analysis a labor-intensive and costly exercise. The incidence of germline mutations in *CDKN2A* in unselected melanoma cases is exceedingly low: it is estimated that only 0.2% of melanoma cases in Australia were due to mutations in *CDKN2A* [8]. On the other hand, the rate of p16 mutations in familial melanoma cases in ethnically diverse populations varies from 8% to 50%. For example, *CDKN2A* mutations were found in 10.3% of a population sample of high risk families in Australia. The wide variation in mutation detection in p16 among melanoma families could reflect the different definitions of "familial melanoma" used in different studies [6,8]. Altogether, 60 different mutations in p16 have been identified to date in melanoma families [9].

The likelihood of finding a mutation in *CDKN2A* is dependent on the number of affected family members overall, rising from 5% in families with two affected members, to 20–40% in families with three or more affected members, to 100% in families with 13 or more affected members. Many of the recurrent mutations in *CDKN2A* are "founder mutations" dating back up to 100 generations [10]. Families with mutations in *CDKN2A* that affect only p14ARF are much less common than mutations that affect p16 with or without affecting p14ARF. Among 30 Israeli melanoma families genotyped for germline p16 mutations, no mutations were found in *CDKN2A* in Ashkenazi Jews (East European origin), which comprised the majority of families in that study. However, a novel missense mutation, 365 G>T, leading to an amino acid change (Gly122Val) was identified in a non-Ashkenazi Jewish family. This mutation causes instability of the p16 protein and impairs the binding of p16 to *CDK4*, impairing its ability to cause a G1 cell cycle arrest in human diploid fibroblasts [11]. Another gene prevalent in the Ashkenazi Jewish population has been found to be associated with ocular melanoma. Germline mutations in *BRCA2* have been studied in patients with ocular melanoma. The prevalence of mutations in this gene was estimated at 3% [12]. The mutation 6174delT in *BRCA2* accounts for a small fraction of Israeli uveal melanoma cases [13]. The estimated gene penetrance for melanoma in individuals with *CDKN2A* mutations by age 80 is 58–92% and is geographically variable [14,15]. Factors affecting the rates of melanoma within the population affect the penetrance among mutation carriers. These risk factors can reflect gene environment or gene-gene interactions. It was postulated that ultraviolet radiation increases the penetrance of *CDKN2A* mutations, being highest in Australia, lower in the U.S., and lowest in Europe [6].

Somatic mutations in *CDKN2A* have been documented in a wide variety of different tumor types. Among a subset of families with germline *CDKN2A* mutations there appears to be an excess of pancreatic cancer, giving p16 mutation carriers a 21.8-fold increased relative risk for developing this tumor type [16]; here the penetrance is estimated at 17% by age 75 for carriers of p16-Leiden 19bp deletion [17]. This risk does not seem to be mutation-specific: among families with *CDKN2A* "founder mutations," some families exhibit pancreatic cancer while some identical mutation carriers do not. There is also an elevated risk of oral squamous cell carcinomas

[6]. Breast cancer has been reported in excess in Swedish melanoma-prone families harboring a founder *CDKN2A* mutation. There is evidence that p14ARF is an independent melanoma predisposition gene. p14ARF inactivation predisposes also to nervous system tumors including astrocytoma, meningioma, schwannoma and neurofibroma [6].

In addition to bona fide pathogenic mutations that are clearly associated with an increased melanoma risk, rare alleles of *CDKN2A* (C500G or C540T polymorphisms in the 3' untranslated region of the gene) are more commonly seen in melanoma patients, and have been significantly associated with risk of developing melanoma in non-familial cases. In addition, carriers of either of these variants had a shorter progression time from diagnosis of the primary tumor to the appearance of metastasis [6,8,18].

CDK4A

The *CDK4A* gene is an oncogene located on chromosome 12q13. Activating mutations in *CDK4* are oncogenic, since the encoded protein product, a kinase, negatively regulates pRb by phosphorylation, thereby causing it to release transcription factors of the E2F family. Thus, overphosphorylation of the Rb protein by the oncogenic version of *CDK4* leads to unregulated transcription via the E2F family of proteins, with resultant uncontrolled cellular proliferation. Oncogenic germline-activating mutations clustered to codon 24 of the *CDK4* gene have been identified in a handful of familial melanoma cases. These mutations undermine the ability of p16 to

The high penetrance genes play a major role in controlling cell division

associate with and inactivate *CDK4* [6]. The increased risk for melanoma development is similar among gene carriers of *CDK4* and *CDKN2A*. These similar penetrance rates can be explained by the fact that both *CDK4* and p16 affect the same downstream effectors (pRb and the genes regulated by the E2F transcription factors) [6].

Low penetrance genes associated with melanoma risk

Melanocortin-1 receptor (MC1R)

MC1R is a "low penetrance susceptibility gene" for cutaneous melanoma [6]. *MC1R* sequence variants are significantly more frequent among melanoma patients than among unaffected healthy controls. Having a *MC1R* sequence variant carries a 2.2 to 3.9-fold risk for developing melanoma. This effect is additive, since carriers of two variants have a 4.1 to 4.8-fold risk [6,19].

Melanocortin-1 receptor plays a role in determining the type of melanin produced by melanocytes. Binding of α -MSH to *MC1R* activates adenylyl-cyclase, resulting in increased intracellular cAMP production and accumulation. This in turn switches melanin production from pheomelanin (yellow-red), which is photosensitive and potentially mutagenic, to eumelanin (brown-black), which is photoprotective [19].

The impact of *MC1R* on melanoma risk was found to be mediated chiefly by the variants associated with “red hair color,” each conferring a 2.2-fold risk of melanoma. In a sample of individuals of British or Irish descent, 53% of those with “red hair color” were found to carry one variant *MC1R* allele and 29% had two variant alleles [19,20]. Only “red hair color” individuals were found to carry two variants. The frequency of a variant allele in blond, brown or black-haired individuals did not exceed 33%. Some *MC1R* sequence variants have been associated with increased risk of melanoma among individuals with dark complexions. There is considerable variation of the *MC1R* gene in European and Asian populations, but very little variation in African populations, indicating the strong negative evolutionary pressure in Africa [21]. Certain *MC1R* gene sequence variants serve as modifiers of the risk for melanoma development and lead to an increase in the penetrance of *CDKN2A* mutations from 50% to 84%, and lower the mean age of onset by 20 years [22].

Epidermal growth factor

EGF has a role in mitogenesis and wound healing. A single nucleotide polymorphism has been found more frequently in melanoma patients compared with unaffected matched controls. Carrying this specific SNP confers a 2.7 relative risk of developing melanoma [6,23].

In this SNP, located 382 nucleotides upstream of the initiation codon of preproEGF, adenine is substituted to guanine. This polymorphism has a functional significance, unlike most SNPs, as it is responsible for determining the level of EGF produced by peripheral blood mononuclear cells cultured *in vitro* after stimulation with phorbol-myristate-acetate and lipopolysaccharide [6]. Cells from individuals homozygous for this SNP produce more EGF compared to cells from heterozygous or individuals homozygous for the common allele. Lastly, individuals who are homozygous had more tumors measuring >3.5 mm by Breslow at presentation.

Glutathione S-transferase M1 gene (GSTM1)

About half of the Caucasian population is genotypically null for the glutathione S-transferase M1 gene (*GSTM1*), which codes for an enzyme that catalyses the detoxification of various compounds, including carcinogenic epoxides. Melanoma patients with red or blond hair are 2.2-fold more likely than controls to be *GSTM1* null, and 9.5-fold more likely to be nullizygous for *GSTM1* and glutathione S-transferase theta 1 (*GSTT1*) [6,24].

CYP2D6

Inactivating polymorphisms at the cytochrome p450 debrisoquine hydroxylase locus (*CYP2D6*), an enzyme involved in detoxifying potentially carcinogenic compounds [6], were found in melanoma patients at significantly higher frequencies. Similarly, a higher frequency of melanoma patients was found to be homozygous for this non-functional allele compared to controls [25].

Vitamin D receptor (VDR)

The vitamin-D receptor ligand, calcitriol, has antiproliferative and pro-differentiation effects on melanocytes and melanoma cells [26]. Polymorphism of the vitamin D receptor gene has been associated with high serum calcitriol levels and increased risk of several types of cancer. The Fok I polymorphism allele, which results in a novel vitamin D receptor translation start site, was found to be significantly more common in melanoma patients compared with unaffected controls [27].

Summary

Malignant melanoma occurs at an increasing rate worldwide. Several genes are involved in malignant melanoma predisposition. These are divided into two major groups – high penetrance and low penetrance genes. Two high penetrance genes have thus far been identified, *CDKN2A* and *CDK4* (cyclin-dependent kinase 4). Both play a major role in controlling cell division; *CDKN2A* is a tumor suppressor gene and *CDK4* is an oncogene. Currently, genetic

A unique mutation in CDKN2A, a high penetrance gene, has been linked to melanoma in non-Ashkenazi Jews

testing for *CDKN2A* in patients in clinical care is still considered premature, since the penetrance of mutations is unknown and knowledge of mutation status would not alter the clinical care [28].

Further characterization of genes contributing to melanoma predisposition will increase our understanding of individuals at risk and how that risk can be modified, enable us to better recognize early-stage disease, and, ultimately, improve treatment strategies leading to reduced morbidity and mortality from melanoma.

References

- Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002;52:23–47.
- Bulliard JL. Site-specific risk of cutaneous malignant melanoma and pattern of sun exposure in New Zealand. *Int J Cancer* 2000;85:627–32.
- Barth A, Wanek LA, Morton DL. Prognostic factors in 1,521 melanoma patients with distant metastases. *J Am Coll Surg* 1995;181:193–201.
- Tucker MA, Goldstein AM. Melanoma etiology: where are we? *Oncogene* 2003;22:3042–52.
- Kopf AW, Hellman LJ, Rogers GS, et al. Familial malignant melanoma. *JAMA* 1986;256:1915–19.
- Hayward N. Genetics of melanoma predisposition. *Oncogene* 2003;22:3053–62.
- Smith-Sorensen B, Hovig E. *CDKN2A* (p16INK4A) somatic and germline mutations. *Hum Mutat* 1996;7:294–303.
- Aitken J, Welch J, Duffy D, et al. *CDKN2A* variants in a population-based sample of Queensland families with melanoma. *J Natl Cancer Inst* 1999;91:446–52.
- Stenson PD, Ball EV, Mort M, et al. The Human Gene Mutation Database (HGMD): 2003 Update. *Hum Mutat* 2003;21:577–81.
- Pollock PM, Spurr N, Bishop T, et al. Haplotype analysis of two recurrent *CDKN2A* mutations in 10 melanoma families: evidence for common founders and independent mutations. *Hum Mutat* 1998;11:424–31.

EGF = epidermal growth factor
SNP = single nucleotide polymorphism

11. Jakobson E, Shemesh P, Azizi E, et al. Two p16 (CDKN2A) germline mutations in 30 Israeli melanoma families. *Eur J Hum Genet* 2000;8:590–6.
12. Scott RJ, Vajdic CM, Armstrong BK, et al. BRCA2 mutations in a population-based series of patients with ocular melanoma. *Int J Cancer* 2002;102:188–91.
13. Iscovich J, Addulrazik M, Cour C, et al. Prevalence of the BRCA2 6174delT mutation in Israeli uveal melanoma patients. *Int J Cancer* 2001;98:42–4.
14. Bishop DT, Demenais F, Goldstein A, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst* 2002;94:894–903.
15. Newton-Bishop JA, Wachsmuth RC, Harland M, et al. Genotype/phenotype and penetrance studies in melanoma families with germline CDKN2A mutations. *J Invest Dermatol* 2000;114:28–33.
16. Goldstein AM, Fraser MC, Struwing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med* 1995;333:970–4.
17. Vasen HFA, Gruis NA, Frantis RR, et al. Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19' deletion of p16 (p16-Leiden). *Int J Cancer* 2000;87:809–11.
18. Kumar R, Smeds J, Berggren P, et al. A single nucleotide polymorphism in the 3'untranslated region of the CDKN2A gene is common in sporadic primary melanomas but mutations in the CDKN2B, CDKN2C, CDK4 and p53 genes are rare. *Int J Cancer* 2001;95:388–93.
19. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet* 1995;11:328–30.
20. Palmer JS, Duffy DL, Box NF, et al. Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? *Am J Hum Genet* 2000;66:176–86.
21. Harding RM, Healy E, Ray AJ, et al. Evidence for variable selective pressures at MC1R. *Am J Hum Genet* 2000;66:1351–61.
22. Box NF, Duffy DL, Chen W, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am J Hum Genet* 2001;69:765–73.
23. Shahbazi M, Pravica V, Nasreen N, et al. Association between functional polymorphism in EGF gene and malignant melanoma. *Lancet* 2002;359:397–401.
24. Kanetsky PA, Holmes R, Walker A, et al. Interaction of glutathione S-transferase M1 and T1 genotypes and malignant melanoma. *Cancer Epidemiol Biomarkers Prev* 2001;10:509–13.
25. Strange RC, Ellison T, Ichii-Jones F, et al. Cytochrome P450 CYP2D6 genotypes: association with hair colour, Breslow thickness and melanocyte stimulating hormone receptor alleles in patients with malignant melanoma. *Pharmacogenetics* 1999;9:269–76.
26. Ranson M, Posen S, Mason RS. Human melanocytes as a target tissue for hormones: *in vitro* studies with 1 α ,25-dihydroxyvitamin D₃, α -melanocyte stimulating hormone, and β -estradiol. *J Invest Dermatol* 1988;91:593–8.
27. Hutchinson PE, Osborne JE, Lear JT, et al. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. *Clin Cancer Res* 2000;6:498–504.
28. Kefford RF, Mann GJ. Is there a role for genetic testing in patients with melanoma? *Curr Opin Oncol* 2003;15:157–61.

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The concept is interesting and well-formed, but in order to earn better than a 'C', the idea must be feasible

A Yale University management professor in response to Fred Smith's paper proposing reliable overnight delivery service. Fred Smith nonetheless founded FedEx in Memphis in 1971, the largest express delivery service in the world.

Capsule

Inhibiting NK cells help pregnancy

The immune system has been thought to present a barrier to the normal development of the fetus through maternal immunity to "foreign" paternal proteins. However, Hibey and colleagues found that efficient activation of innate natural killer (NK) cells may actually help fetal growth. The multiple receptors carried by NK cells exist in two basic forms that lead to either cellular activation or inhibition. It is the balance between these signals that ultimately dictates the NK cell's response. Pregnant women carrying particular combinations of receptor genes and their polymorphic ligands that favor NK cell inhibition were more likely

to develop the potentially lethal condition preeclampsia, in which placental blood flow becomes impaired. Thus, uterine NK cells and their cytokines may promote the trophoblast remodeling of the placental spiral arteries that generates the essential increase in blood flow required during placenta formation. Thus, by inhibiting, rather than activating NK cells, it is possible that this remodeling becomes less efficient and sets the stage for preeclampsia.

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