

Genetic Epidemiology of Cutaneous Melanoma



A Global Perspective

Alisa M. Goldstein, PhD; Margaret A. Tucker, MD

Cutaneous malignant melanoma (CMM) results from the interaction of genetic, host, and environmental influences. Epidemiologic studies of melanoma have shown that the major environmental risk factor is UV radiation. The exposure-response relationship, however, seems complex, with intermittent sun exposure likely to be more important for risk than total lifetime exposure (for a review, see Armstrong and English¹). The host factors most strongly associated with melanoma are melanocytic nevi (moles), both clinically banal and atypical (dysplastic).^{1,2} Other host factors implicated in melanoma include fair hair color, light eye color, increased freckling, and an inability to tan. Nonmelanoma skin cancer (basal cell or squamous cell carcinoma) also increases the risk for melanoma.¹

Approximately 5% to 12% of malignant melanomas develop in individuals who have 1 or more first-degree relatives with CMM.³ Such clustering is denoted familial melanoma. No precise definition exists for the term *familial melanoma*; its use varies across studies and geographic locations. In general, though, familial melanoma is clinically and histologically indistinguishable from nonfamilial melanoma. However, there are generally differences in age at diagnosis, lesion thickness, and frequency of multiple lesions.^{4,5} Familial melanoma has an earlier age at diagnosis, thinner CMM tumors, and a higher frequency of multiple lesions than nonfamilial. Some of the familial melanoma clusters occur by chance. Others may occur because family members share the same host characteristics such as hair and eye color, freckling, and skin type. Familial clustering is higher in more heavily insolated areas such as Australia. Thus, clustering likely results from both genetic and shared nongenetic factors with only a subset of patients with familial melanoma likely having inherited a mutation in a melanoma susceptibility gene.⁶ The proportion of such cases in the

population is unknown, although it is believed to be at least a few percent.

MELANOMA PREDISPOSITION GENES

To date, 2 melanoma predisposition genes have been identified. Both are inherited in an autosomal dominant pattern showing vertical transmission of the disease and similar numbers of affected men and women. The first gene, *CDKN2A*, is located on the short arm of chromosome 9 (9p21).^{7,8} It is a complex tumor suppressor gene that encodes 2 distinct proteins, p16 and p14^{ARF}. The p16 protein, comprising exons 1 α , 2, and 3, is a cell cycle regulatory protein that inhibits the activity of the cyclin D1–cyclin-dependent kinase 4 (CDK4) or 6 (CDK6) complex. p16 Negatively regulates cell growth by arresting cells at G₁ phase of the cell cycle.⁹ The second protein, p14^{ARF}, is produced from alternate reading frames (ARFs) and comprises exons 1 β , 2, and 3. p14^{ARF} Acts via the p53 pathway to induce cell cycle arrest or apoptosis.^{10,11}

In contrast, the second identified melanoma susceptibility gene, *CDK4*, maps to the long arm of chromosome 12 (12q13).^{12,13} It seems to function as an oncogene that is resistant to the normal physi-

From the Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Md.

ological inhibition of p16. To date, cosegregating germline mutations have been detected in only 3 melanoma-prone families worldwide.^{14,15} Thus, *CDK4* mutations are presumed to be very rare. Although the dominant oncogene *CDK4* and tumor suppressor *CDKN2A* have different mechanisms of action, clinical characteristics such as age at diagnosis, numbers of melanoma tumors, and number of nevi do not differ between melanoma patients from *CDKN2A* vs *CDK4* families.¹⁶ Such comparisons have limited power, however, because of the small numbers of melanoma cases with *CDK4* mutations. Direct assessment of *CDK4* families will remain limited because of their rarity. Other genetic factors and their inheritance patterns remain to be determined.

CDKN2A

Germline mutations in the major known melanoma susceptibility gene *CDKN2A* have been found in melanoma-prone families from North America, Europe, and Australasia. Overall, *CDKN2A* mutations have been found in approximately 20% of melanoma-prone families with 3 or more members who have the disease from around the world.³ The frequency of detected *CDKN2A* mutations varies considerably across different geographic areas but is directly related to the numbers of melanoma patients per family. The frequency of mutations increases as the number of melanoma cases in the family increases. For example, the frequency of detectable mutations is lower than 5% for families with only 2 members with melanoma. It increases to higher than 50% for families with more than 6 melanoma cases.^{3,17} The occurrence of multiple CMM lesions in an individual also increases the chances of finding a *CDKN2A* mutation. Approximately 10% of patients with multiple primary melanoma tumors, but without family histories of the disease, have been shown to carry germline mutations in the *CDKN2A* gene.¹⁷⁻¹⁹ The absence of a positive family history in these patients, however, suggests that other factors, genetic and/or environmental, affect the phenotypic expression of *CDKN2A* mutations. The occurrence of multiple primary melanoma tumors in a patient with a family history of CMM also increases the frequency of detecting a *CDKN2A* mutation.^{15,19,20}

Most mutations described to date are missense mutations (ie, mutations that cause a change from one amino acid to another) scattered throughout the *CDKN2A* coding region. A mutation in the 5' untranslated region (G34T) has also been described.²¹ Some mutations have been observed only once, while others have repeatedly been found in different families. Repeat mutations may occur by chance or because the mutation occurs in a region of the gene that is prone to change (ie, a mutation hot spot) or because the mutation resulted from a single genetic origin (and thus all recurrences derive from that original ancestor). Analyses of common recurrent *CDKN2A* mutations studied to date from the same geographic areas (eg, 225del19 from the Netherlands, 113insR from Sweden, G34T from Canada) or from geographically diverse areas (eg, M53I, 23ins24, G101W) showed that most seem to have originated from a common founder or ancestor.^{16,21-25} In fact, only 1 recurrent

mutation, 23ins24, a 24-base pair duplication, has been shown to have multiple origins, probably because of the inherent instability of the 5' tandem repeat region.²⁴

Once a mutation has been shown to be a founder mutation, it is possible to estimate the age at which the mutation originated. Such calculations have been conducted for 2 recurrent mutations, G101W and 113insR^{23,25}; both mutations seem to have arisen approximately 100 generations ago, although the confidence intervals for these estimates are very wide. Given a remote origination, one would hypothesize that both mutations should be geographically dispersed. G101W has been found in Australia, North America, and Europe with a particularly high frequency in southwestern Europe.²⁵ In contrast, 113insR has been identified primarily in Sweden; this observation has led to the hypothesis that the *CDKN2A* locus lies in a recombination hot spot and that the number of generations that have passed from the original founder may represent an overestimate.²³ Future studies will need to further examine this interesting question.

CDKN2A is a susceptibility gene, but its inherited effects may not be limited to melanoma. The most consistent association occurs with pancreatic cancer. Mutations in *CDKN2A* seem to be associated with an increased risk of pancreatic cancer in a subset of families,²⁶⁻²⁹ but the precise relationship between alterations in the *CDKN2A* gene and pancreatic cancer remains unknown. No relationship between specific mutations and pancreatic cancer has yet been found. It is also not possible to predict what genotype or phenotype predisposes an individual to pancreatic cancer in families with *CDKN2A* mutations. These observations have led to the hypothesis that the inconsistent occurrence of pancreatic cancer cannot be explained by the *CDKN2A* mutation itself, but is likely due to the influence of other factors, genetic and/or environmental.^{16,17,27}

Other cancers have also been found in melanoma-prone *CDKN2A* families, but usually in too few numbers to test the association. Recently, investigators from Sweden reported a significant excess of breast cancer among Swedish *CDKN2A* families carrying the Swedish founder mutation 113insR.²⁹ Future studies will be needed to determine whether this breast cancer excess is mutation specific or whether genes inherited from both maternal and paternal sides of certain families increase disease risk/expression or modify the tumor phenotype.

The rare occurrence of melanoma with nervous system tumors, commonly astrocytoma (MIM 155755), has also been associated with the chromosome 9p21 region. Germline deletions of the genes encoding p16 and p14^{ARF} have been detected in one kindred (6 patients with CMM and 7 with nervous system tumors, including 4 with both) and the whole p16, p14^{ARF}, and p15 cluster in a second (7 patients with CMM and 3 with nervous system tumors, including 2 with both).³⁰ In addition, a 3-generation family with 2 cases of melanoma, 2 cases of both CMM and glioblastoma multiforme, and 1 case of astrocytoma had a hemizygous germline deletion of p16/p14^{ARF}.³¹ The germline deletions in the 3 families mentioned above suggested either that the

multiple cancer susceptibility resulted from inactivation of contiguous tumor suppressor genes or that the deletion specifically targeted p14^{ARF} (exon 1 β).^{20,31} Recently, a 3-generation family displaying the melanoma-astrocytoma syndrome was found to have a germline deletion of the p14^{ARF}-specific exon 1 β ; p16 and p15 were not affected. The finding suggests that either loss of p14^{ARF} function is the critical abnormality for the melanoma-astrocytoma syndrome rather than contiguous loss of both p16/p14^{ARF} and p15, or that expression of p16 is disrupted by unknown mechanisms.³²

CDKN2A: FUTURE STUDIES

The relationship between melanoma, *CDKN2A* mutations, and other tumors has yet to be fully determined. In addition, although *CDKN2A* mutations confer substantial risk for melanoma (and other tumors), many mutation carriers do not develop melanoma.³ Unaffected individuals homozygous for a *CDKN2A* mutation have also been identified.²² These studies suggest that another gene or multiple genes and/or environmental factors are involved in the pathogenesis of melanoma and in determining the phenotypic expression of the trait among gene carriers (penetrance). Also, although *CDKN2A* mutations confer increased risk for melanoma, clinicoepidemiologic variables such as dysplastic or atypical nevi, total numbers of nevi, and solar injury have been shown to further enhance the disease risk among mutation carriers.³ Future studies are needed to estimate penetrance for melanoma and possibly other tumors and assess the relationship between *CDKN2A* and modifying genetic and/or environmental factors that may influence disease expression. Population-based studies are also needed to determine the frequency of *CDKN2A* mutations around the world and its impact on the total melanoma burden.

FUTURE GENETIC EPIDEMIOLOGIC RESEARCH

The 2 identified melanoma susceptibility genes, *CDKN2A* and *CDK4*, account for only 20% to 40% of the inherited forms of melanoma¹⁷; other genetic factors and their inheritance patterns remain to be discovered. Cytogenetic and loss of heterozygosity studies have consistently suggested the possibility of additional tumor suppressor genes on chromosome 9p (for a review, see Pollock et al³³). In addition, although most melanoma-prone families that show strong evidence of linkage to chromosome 9p have *CDKN2A* mutations, a subset of these families does not have detectable mutations. Whether these families have noncoding region mutations or large deletions or alternative mechanisms of inactivation (eg, methylation) or whether another or multiple other chromosome 9p melanoma susceptibility genes located near *CDKN2A* exist needs further exploration.

The search for additional melanoma susceptibility genes will be strongly influenced by the overall frequency of mutations in these genes. For example, cytogenetic, loss of heterozygosity, and linkage studies led to the localization of a melanoma susceptibility gene to chromosome 9p21, the region where *CDKN2A* is located.³ The discovery that *CDK4* was a melanoma sus-

ceptibility gene used candidate gene approaches.^{12,14} Mutation testing and sequencing of candidate genes, genotyping studies with subsequent linkage and loss of heterozygosity analyses, cytogenetic studies, and proteomic studies should all be useful in the identification of additional susceptibility genes. And once additional melanoma predisposition genes are discovered, the same questions that are now being asked for *CDKN2A* (eg, penetrance, modifying genetic and/or environmental factors, associations with other tumors) will need to be explored.

MUTATION TESTING

Current gaps in the understanding of the phenotypic expression of melanoma susceptibility genes in families and in the general population mean that DNA mutation testing should not be used as a guide in the clinical practice of prevention and surveillance. All individuals considered to be at high risk of melanoma, based on the well-established melanoma risk factors, should be managed using the same approach.¹⁷

The American Society of Clinical Oncology statement on genetic testing for cancer susceptibility recommends that genetic mutation testing be performed only when "the tests can be adequately interpreted; and the results will influence the medical management of the patient or family member."³⁴ The Melanoma Genetics Consortium, made up of familial melanoma research groups from the United States, Europe, and Australia, reviewed current information about genetic testing and concluded that neither of these criteria is currently met for the testing of *CDKN2A* and *CDK4*.¹⁷ The consortium concluded that it is therefore premature to offer mutation testing outside of defined research protocols except in rare circumstances, and then only after careful genetic counseling that adequately addresses the low likelihood of finding mutations, current uncertainties about the risk/expression of specific mutations, and the potential benefits and risks of positive and negative results of genetic testing. The consortium plans to review this advice regularly to keep up-to-date with developments in the field and to maintain a current consensus opinion.¹⁷

CONCLUSIONS

Cutaneous melanoma has a complex etiologic context resulting from the interrelationships of genetic, host, and environmental factors. Genetic epidemiologic studies are useful for identifying susceptibility genes, exploring associations with other tumors, examining gene-gene and gene-environment interactions, and discovering genetic and/or environmental influences that modify the disease expression. Better understanding of these diverse factors should lead to improved prevention, detection, and treatment options.

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Corresponding author and reprints: Alisa M. Goldstein, PhD, Genetic Epidemiology Branch/National Cancer Institute, Executive Plaza South, Room 7004, 6120 Executive Blvd, MSC 7236, Bethesda, MD 20892-7236 (e-mail: goldstea@exchange.nih.gov).

1. Armstrong BK, English DR. Cutaneous malignant melanoma. In: Schottenfeld D, Fraumeni JF Jr, eds. *Cancer Epidemiology and Prevention*. New York, NY: Oxford University Press; 1996:1282-1312.
2. Tucker MA, Halpern A, Holly EA, et al. Clinically recognized dysplastic nevi: a central risk factor for cutaneous melanoma. *JAMA*. 1997;277:1439-1444.
3. Goldstein AM, Tucker MA. Familial melanoma and its management. In: Eeles R, Easton D, Eng C, Ponder B, eds. *Genetic Predisposition to Cancer*. 2nd ed. London, England: Edward Arnold Publishers Ltd; 2001. In press.
4. Barnhill RL, Rousch GC, Titus-Ernstoff L. Comparison of nonfamilial and familial melanoma. *Dermatology*. 1992;184:2-7.
5. Kopf AW, Hellman LJ, Rogers GS, et al. Familial malignant melanoma. *JAMA*. 1986;256:1951-1959.
6. Aitken J, Duffy D, Green A, et al. Heterogeneity of melanoma risk in families of melanoma patients. *Am J Epidemiol*. 1994;140:961-973.
7. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science*. 1994;264:436-440.
8. Nobori T, Miura K, Wu DJ, et al. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*. 1994;368:753-756.
9. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*. 1993;366:704-707.
10. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell*. 1998;92:725-734.
11. Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell*. 1998;92:713-723.
12. Wolfel T, Hauer M, Schneider J, et al. A p16INK4a insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science*. 1995;269:1281-1284.
13. Demetrick DJ, Zhang H, Beach DH. Chromosomal mapping of human CDK2, CDK4, and CDK5 cell-cycle kinase genes. *Cytogenet Cell Genet*. 1994;66:72-74.
14. Zuo L, Weger J, Yang Q, et al. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet*. 1996;12:97-99.
15. Soufir N, Avril M-F, Chompret A, et al. Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France. *Hum Mol Genet*. 1998;7:209-216.
16. Goldstein AM, Struewing JP, Chidambaram A, et al. Genotype-phenotype relationships in American melanoma-prone families with *CDKN2A* and *CDK4* mutations. *J Natl Cancer Inst*. 2000;92:1006-1010.
17. Kefford RF, Newton-Bishop JA, Bergman W, et al. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: a consensus statement of the Melanoma Genetics Consortium. *J Clin Oncol*. 1999;17:3245-3251.
18. Monzon J, Liu L, Brill H, et al. *CDKN2A* mutations in multiple primary melanomas. *N Engl J Med*. 1998;338:879-887.
19. MacKie RM, Andrew N, Lanyon WG, Connor JM. *CDKN2A* germline mutations in U.K. patients with familial melanoma and multiple primary melanomas. *J Invest Dermatol*. 1998;111:269-272.
20. Holland EA, Schmid H, Kefford RF, et al. *CDKN2A* (p16INK4a) and *CDK4* mutation analysis in 131 Australian melanoma probands: effect of family history and multiple primary melanomas. *Genes Chromosomes Cancer*. 1999;25:1-10.
21. Liu L, Dilworth D, Gao L, et al. Mutation of the *CDKN2A* 5' UTR creates an aberrant initiation codon and predisposes to melanoma. *Nat Genet*. 1999;21:128-132.
22. Gruis NA, van der Velden PA, Sandkuijl LA, et al. Homozygotes for *CDKN2* (p16) germline mutation in Dutch familial melanoma kindreds. *Nat Genet*. 1995;10:351-353.
23. Hashemi J, Bendahl P-O, Sandberg T, et al. Haplotype analysis and age estimation of the 113insR *CDKN2A* founder mutation in Swedish melanoma families. *Genes Chromosomes Cancer*. 2001;31:107-116.
24. 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;11:424-431.
25. Ciotti P, Struewing JP, Mantelli M, et al. A single genetic origin for the G101W *CDKN2A* mutation in 20 melanoma-prone families. *Am J Hum Genet*. 2000;67:311-319.
26. Goldstein AM, Fraser MC, Struewing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med*. 1995;333:970-974.
27. Bergman W, Gruis N. Familial melanoma and pancreatic cancer [letter]. *N Engl J Med*. 1996;334:471.
28. Ghiorzo P, Ciotti P, Mantelli M, et al. Characterization of Ligurian melanoma families and risk of occurrence of other neoplasia. *Int J Cancer*. 1999;83:441-448.
29. Borg A, Sandberg T, Nilsson K, et al. High frequency of multiple melanomas and breast and pancreas carcinomas in *CDKN2A* mutation-positive melanoma families. *J Natl Cancer Inst*. 2000;92:1260-1266.
30. Bahuau M, Vidaud D, Jenkins RB, et al. Germ-line deletion involving the *INK4* locus in familial proneness to melanoma and nervous system tumors. *Cancer Res*. 1998;58:2298-2303.
31. Tachibana I, Smith JS, Sato K, Hosek SM, Kimmel DW, Jenkins RB. Investigation of germline PTEN, p53, p16^{INK4A}/p14^{ARF}, and CDK4 alterations in familial glioma. *Am J Med Genet*. 2000;92:136-141.
32. Randerson-Moor JA, Harland M, Williams S, et al. A germline deletion of p14^{ARF} but not CDKN2A in a melanoma-neural system tumor syndrome family. *Hum Mol Genet*. 2001;10:55-62.
33. Pollock PM, Welch J, Hayward NK. Evidence for three tumor suppressor loci on chromosome 9p involved in melanoma development. *Cancer Res*. 2001;61:1154-1161.
34. American Society of Clinical Oncology. Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility. *J Clin Oncol*. 1996;14:1730-1740.