Influence of Genes, Nevi, and Sun Sensitivity on Melanoma Risk in a Family Sample Unselected by Family History and in Melanoma-Prone Families

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Background: Few family studies have investigated the effects of genetic, environmental, and host factors on melanoma risk, and most have been restricted to high-risk families. We assessed the role of these factors on melanoma risk in two types of families: families ascertained through melanoma probands but unselected by family history and melanoma-prone families. Methods: Data on pigmentary traits, nevus phenotypes, exposure to sun, and reactions to sunlight were collected from 295 families unselected by family history and 53 melanoma-prone families. We modeled melanoma risk using a logistic regressive model incorporating the effect of a melanoma-predisposing gene, familial dependence, and potential risk factors (e.g., pigmentary traits, nevus phenotypes, history of sun exposure, skin reactions to sunlight). Maximum-likelihood estimates of the parameters of the regressive model were used to compute odds ratios associated with each risk factor and age-specific melanoma risk depending on the genotype at the melanoma-predisposing gene and the effects of risk factors. All statistical tests were two-sided. Results: In the families unselected by family history, there was statistically significant evidence (P<.001) for a dominant gene, with melanoma risk reaching 0.49 and 0.67 by age 80 years in male and female gene carriers, respectively. Melanoma risk was statistically significantly influenced by total nevi (odds ratio of hazard function [OR] = 5.81, 95% confidence interval [CI] = 3.47 to 8.99), sun exposure (OR = 5.37, 95% CI = 4.44 to 6.36), and sunburn interacting with the gene (OR = 26.31, 95% CI = 7.56 to 99.22 in gene carriers and OR = 1.67, 95% CI = 1.36 to 2.03 in noncarriers). Twenty of the 53 melanoma-prone families had cosegregating mutations in CDKN2A, a gene known to be associated with melanoma. In these 53 families, three risk factors in addition to CDKN2A mutations increased melanoma risk: dysplastic nevi (OR = 2.32, 95% CI = 2.08 to 2.58), total nevi (OR = 1.99, 95% CI = 1.61 to 2.20) and sunburn (OR = 5.16, 95% CI = 4.82 to 5.52). Conclusions: Together, a melanoma-predisposing gene (identified as being CDKN2A in melanoma-prone families), number of nevi and/or dysplastic nevi, and sun-related covariates influence melanoma risk in both families unselected by family history and melanoma-prone families. [J Natl Cancer Inst 2004;96:785–95]

The etiology of cutaneous malignant melanoma is heterogeneous and complex. Approximately 10% of malignant melanomas occur in a familial setting, and susceptibility to melanoma is associated with increased incidence of nevi or dysplastic nevi in some families (1,2). Segregation analyses of melanoma have suggested autosomal dominant inheritance (3) or a more complex genetic mechanism of melanoma susceptibility (4,5). To date, mutations in two genes, CDKN2A and CDK4, have been shown to confer a high risk of melanoma. CDKN2A encodes two distinct proteins translated in alternate reading frames from alternatively spliced transcripts. The alpha transcript encodes the p16INK4A protein, which inhibits the activity of the cyclin D1-CDK4 complex, preventing the cell from progressing through the G1 cell cycle checkpoint and therefore acting as a tumor suppressor (6,7). The beta transcript encodes the p14ARF protein, which acts by way of the p53 pathway to induce cell cycle arrest or apoptosis (8). Most germline CDKN2A mutations affect the p16INK4A protein, and such mutations have been observed in approximately 20% (range = <5% to >50%) of tested melanoma-prone families from Australia, Europe, and North America (9,10). CDK4 acts as an oncogene, and germline mutations of this gene have been detected in only three melanoma-prone families worldwide (9,11). In addition to CDKN2A and CDK4, which have been detected in only high-risk families, common variants of the melanocortin-1 receptor gene, MC1R, which plays a key role in human skin pigmentation, have been shown to be associated with a low risk of melanoma (12–14). Other genetic factors remain to be identified.

Epidemiologic studies (for review, see (15)) suggest that exposure to sunlight is the major environmental risk factor associated with the development of melanoma, although the exposure–response relationship appears to be complex. The major host factors associated with melanoma are high numbers of melanocytic nevi, both clinically banal nevi and atypical (i.e., dysplastic) nevi (16,17). Other host factors associated with melanoma include hair color, eye color, skin color, and skin reactions to sun exposure (18,19).

Few family studies have investigated the joint effects of genetic, environmental, and host factors on melanoma risk and most such studies have been restricted to high-risk families. Analysis of the relationship between CDKN2A mutation status and three risk factors (total nevi, dysplastic nevi, and solar injury) in 20 North American melanoma-prone families showed that numbers of total nevi and of dysplastic nevi were associated with increased melanoma risk beyond the risk increase associated with CDKN2A mutations (20). Examination of the pen...
dentrance of CDKN2A mutations in 80 multiple-case families from Europe, Australia, and the United States by the Melanoma Genetics Consortium has indicated that penetrance varies with geographic location (21). Moreover, variants of the MC1R gene have been shown to modify melanoma risk in multiple-case families segregating CDKN2A mutations (14,22). As recently discussed for breast cancer (23,24), which shows patterns of familial aggregation similar to those seen with melanoma, it is important to carefully identify the different factors modifying the penetrance of cancer predisposing mutations at the population level and in different familial settings to develop effective preventive strategies.

To explore the role of genetic and other risk factors in the etiology of melanoma, we conducted an epidemiologic and genetic study to investigate the effects of genetic, environmental (i.e., sun exposure), and host factors (pigmentary characteristics, nevus phenotypes, skin reactions to sun exposure) on melanoma risk. We examined risk in both families ascertained through melanoma probands but unselected by family history and in families who are melanoma prone.

PARTICIPANTS AND METHODS

Ascertainment of Families and Data Collection

Families unselected by family history were ascertained through 295 melanoma patients who were followed in the Department of Dermatology at the Institut Gustave Roussy from January 1, 1986 to December 31, 1989. Inclusion criteria included histologically confirmed cases of melanoma in white patients who had been living in France for more than 10 years. Ninety-nine percent of the patients who were approached about participation in the study agreed to participate. Most patients were newly diagnosed with melanoma, and the remaining patients had been recently diagnosed cases and were undergoing follow-up. These 295 patients did not differ from all melanoma patients seen at the Institut Gustave Roussy during the same time period in terms of age, sex, and clinical and histologic characteristics of melanoma (25).

We identified extended melanoma-prone families, i.e., families with at least two melanoma cases (one proband plus one affected individual among relatives up to a third degree of relationship), following the same inclusion criteria as described above. These families were ascertained from the Institut Gustave Roussy and other French hospitals forming the French Familial Melanoma Study Group. From a total sample of 100 melanoma-prone families collected since 1986, there were 53 families eligible (according to the criteria above and having DNA available for testing among affected first- or second-degree relatives) for CDKN2A mutation testing.

For both types of families, family data, including demographic characteristics (sex, date and place of birth, and if deceased, age at and cause of death), occurrence of melanoma and any other cancer, and age at diagnosis, were collected by interviewing the probands about their first-, second-, and third-degree relatives. Melanoma diagnosis was confirmed by medical records, review of pathologic material, and/or pathology reports. A trained dermatologist carried out physical examinations to determine the total number of nevi, which was classified into three categories (<10, 10–50, >50 nevi), and the possible presence of atypical moles (moles ≥5 mm in diameter with irregular margins and variegated color) in all probands and relatives who came to the hospital for the purpose of the study. Individuals who were not seen in the hospital were asked to report their total number of nevi (according to the three categories mentioned above) on a questionnaire. A pilot study in a subset of 50 relatives indicated good agreement between the self-reported nevus count and the one assessed by physical examination (26).

A questionnaire recording data on various risk factors was completed by the probands and relatives seen at the hospital and was distributed by probands to all other relatives. A total of 90% of the probands’ relatives from the families unselected by family history answered the questionnaire by mail, whereas 70% of the probands’ relatives belonging to the melanoma-prone families were examined by a dermatologist. The data recorded on the questionnaire for each family member included skin color (pale or dark); eye color (pale or dark); hair color (red, blonde, light brown, dark brown, or dark), presence (or not) of freckles and café au lait spots; number of nevi (three categories defined above); presence of atypical/dysplastic nevi (only in those individuals examined by a dermatologist); degree of exposure to sunlight (low, medium, or high) during their vacations, leisure time, and work time; artificial ultraviolet (UV) exposure (yes or no); long stay (>1 year) in a sunny country; skin reactions to sunlight evaluated by the ability to tan (low, medium, or high); and propensity to sunburn (low, medium, or high).

Written informed consent was obtained from all probands and family members participating in the study under an institutional review board-approved protocol. For the families unselected by family history, data on potential risk factors were obtained for all probands, 90% of living first-degree relatives (except for the presence of dysplastic nevi, which was missing in most individuals and was therefore omitted from the analyses), and 50% of living second-degree relatives. Thus, segregation analysis of this family sample was restricted to the probands and their first-degree relatives, including siblings and parents. For the melanoma-prone families, information on risk factors was obtained in at least 70% of all living relatives.

Laboratory Methods

CDKN2A gene mutations were identified in genomic DNA extracted from peripheral blood lymphocytes using a two-stage process involving an initial screen for heterozygous bases with single-stranded conformational polymorphism (SSCP) analysis followed by direct sequencing of the polymerase chain reaction (PCR) products displaying abnormal migration patterns, as described previously (9). In brief, each of the three CDKN2A exons was amplified by PCR from genomic DNA using the appropriate primers. For SSCP, PCR products were diluted and electrophoresed on two Hydrolink MDE gels (FMC Bioproducts, Rockland, ME), with either 8% glycerol at room temperature or without glycerol at +4°C. Gels were run at 8 W, either for 14 h at room temperature or for 12 h at 4°C, dried, and autoradiographed. Samples displaying abnormal migration patterns were subsequently sequenced on both strands, by either manual or automated methods using the PCR primers. PCR products were sequenced manually with a T7 sequencing kit (Pharmacia Biotech, Uppsala, Sweden) or with the Dye Terminator Cycle sequencing Ready Reaction kit or the ABI prism™ dRhodamine Terminator Cycle sequencing Ready Reaction kit.
Statistical Analysis

Regressive model for segregation and combined segregation–linkage analyses. Analysis of the familial transmission of melanoma (i.e., segregation analysis) in the families unselected by family history and analysis of the cotransmission of melanoma and CDKN2A mutation status (i.e., combined segregation–linkage analysis) in the melanoma-prone families were performed using a logistic regression model (27) extended to take into account variable age at diagnosis of disease (28) and linked marker loci (29). The regression model, which is formally known as the class D regressive model, allows the construction of patterns of correlations within families to include both genetic and nongenetic factors. This model is constructed by specifying a regression relationship between each individual and nongenetic factors. This model is constructed by specifying a regression relationship between each individual’s phenotype (i.e., affected or unaffected with melanoma) and a set of explanatory variables, including the individual’s genotype (at a putative melanoma-predisposing gene in the segregation analysis or at the CDKN2A locus in the combined segregation–linkage analysis), the phenotypes of older relatives (to account for residual familial correlations), and covariates, such as the individual’s age, sex, pigmented traits, nevus phenotypes, and sun-related covariates (history of sun exposure, skin reactions to sunlight).

Under the regressive model, the probability of observing a family with a particular configuration of affected and unaffected individuals, termed the “likelihood,” is written as the product of the probability of the vector of genotypes at the melanoma-predisposing locus multiplied by the penetrance function summed over all unobserved disease genotypes in family members (segregation analysis). In the combined segregation–linkage analysis, in which CDKN2A is assumed to be the melanoma-predisposing gene, the summation is only over individuals with an unknown CDKN2A genotype. For individuals who have no ancestors in the pedigrees (i.e., founders of the pedigrees and spouses), the probability of unobserved disease genotypes is expressed in terms of the frequency of the putative deleterious allele or the frequency of CDKN2A mutations in the general population; for those with ancestors in the pedigrees this probability is expressed in terms of Mendelian probabilities [and general transmission probabilities (30) in segregation analysis]. The frequency of CDKN2A mutations in the population was assumed to be .0001, as explained in the analysis of the Melanoma Genetics Consortium data (21).

The penetrance function (probability of disease phenotype \(Y\), given the vector of genotypes \(g\), antecedents’ phenotypes \(Y_A\), and covariates \(X\)) over \(n\) individuals in the family is decomposed in a product of penetrance functions for each individual, \(i\):

\[
P(Y|g, Y_A, X) = \prod_{i=1}^{n} P(Y_i|g_i, Y_{Ai}, X_i),
\]

where \(g_i\) is the \(i\)th individual’s genotype, \(Y_{Ai}\) is the vector of the antecedents’ phenotypes of \(i\) and \(X_i\) is the vector of covariates for \(i\).

Survival analysis concepts were introduced to take into account a censored age at diagnosis of melanoma (28). Age at diagnosis is regarded as a failure time, and age at examination (for unaffected family members) as a censored failure time, in which the scale for measuring time is age. Because no individuals under the age of 5 years were affected with melanoma in the families unselected by family history and no individuals under the age of 15 years were affected in the melanoma-prone families, the period of follow-up was taken from either 5 years or 15 years of age (depending on the sample) to age at diagnosis for affected patients, age at examination for unaffected family members (or affected patients with unknown age at diagnosis), or age at death for deceased family members. This follow-up period was partitioned into \(K\) mutually exclusive intervals of 1 year each (1…\(K\)). In each interval, we calculated the hazard function \(\lambda(k)\), which is the probability of being affected in the \(k\)th interval given not being affected before. The penetrance function is then derived from the hazard function \(\lambda(k)\). For affected patients, the penetrance function is a density function evaluated at that individual’s actual age of diagnosis \(k\):

\[
f(k) = \lambda(k) \prod_{h=1}^{k-1} [1 - \lambda(h)].
\]

For unaffected family members, the penetrance function is the probability of being unaffected at all ages up to the current age \(k\):

\[
S(k) = \prod_{h=1}^{k} [1 - \lambda(h)].
\]

For patients with an unknown age at diagnosis but a known age at examination, \(k\), the penetrance function becomes \(F(k) = 1 - S(k)\). The penetrance function is defined as 1 for individuals with unknown disease status. The hazard function \(\lambda_i(k)\), for the \(i\)th individual in the \(k\)th interval, is a logistic function: 

\[
\lambda_i(k) = \exp\{\theta_i(k)\}/(1 + \exp(\theta_i(k)));
\]

where \(\theta_i(k)\), the logit of the hazard function, is as follows:

\[
\theta_i(k) = \alpha_{gi} + \sum_{j=1}^{i-1} \Gamma_{ji} Y_j + \beta_{gji} X_i (k) + \nu_{gi}(k).
\]

The parameter \(\alpha_{gi}\) is the genotype-specific baseline parameter; \(\Gamma_{ji}\) is the row vector of regression coefficients on \(j\) antecedents’ phenotypes of the \(i\)th individual; \(\beta_{gji}\) is the row vector of genotype-specific regression coefficients for covariates \(X(k)\) that can be time dependent; \(\nu_{gi}(k)\) is a function of \(k\) that represents the variation of the logit of the hazard function with time and can be genotype-dependent. In its general form, the class D regressive model specifies four types of familial dependence of the \(i\)th person on antecedents that were taken into account in segregation analysis of the families unselected by family history: spouse (\(\Gamma_{Si}\)), father (\(\Gamma_{Fi}\)), mother (\(\Gamma_{Mi}\)), and preceding siblings (\(\Gamma_{Ci}\)). In melanoma-prone families with CDKN2A mutations, CDKN2A was assumed to be the only cause of familial aggregation of melanoma (\(\Gamma_{mi}\) parameters set to zero), whereas in those high-risk families without CDKN2A mutation, vertical transmission of melanoma was accounted for by one set of \(\Gamma_{pi}\) parameters (\(\Gamma_{Fi} = \Gamma_{Mi} = \Gamma_{Pi}\)), modeling the dependence of \(i\)th individual on parents. The hazard function can be assumed to be constant over time \([\nu_{gi}(k) = 0]\) or varying with time using different parametric functions of \(k\). We found that the function \(\nu_{gi}(k) = \delta_{gi} \times \ln(k)\) fit the data better than a polynomial function of \(k\) (linear, quadratic or cubic). The function \(\nu_{gi}(k) = \delta_{gi} \times \ln(k)\) was then used in all analyses. 
Risk factors considered by segregation and combined segregation–linkage analyses. To limit the total number of parameters in the regressive model to be estimated in segregation analysis, which is highly time consuming (in terms of computer time) when it includes a correction for ascertainment of the families through probands, we considered in this analysis only those risk factors that were found to be statistically significant in a preliminary analysis that compared all potential risk factors between melanoma probands and either spouses (111 probands compared with 111 spouses) or unaffected siblings (85 probands compared with 85 unaffected siblings closest in age with the probands). These comparisons showed that several factors were statistically significantly associated with melanoma: history of sun exposure (high versus low or medium), propensity to sunburn (high versus low or medium), and total nevi (>50 nevi versus ≤50 nevi).

All potential risk factors for which information was collected (skin, eye, and hair color; total nevi; presence of dysplastic nevi; history of sun exposure; propensity to sunburn; ability to tan) were considered in the combined segregation–linkage analysis of the melanoma-prone families. These risk factors were all dichotomized, with the baseline versus the at-risk categories being defined as follows: hair color (dark or dark brown versus light brown, blonde, or red); eye color (dark versus pale); skin color (dark versus pale); propensity to sunburn (low or medium versus high); ability to tan (high or medium versus low); sun exposure (low or medium versus high); total nevi (≤50 versus >50 nevi); and dysplastic nevi (absent versus present). To deal with missing data on risk factors, we used the missing indicator method (31), which created two dummy variables for each risk factor—that is, a missing-value indicator set equal to 1 for missing/unknown and 0 for known and a second variable set equal to 1 for exposed and 0 for unexposed or unknown. This method has been found to provide similar results to the complete case method (20,32) and has the advantage of keeping all individuals in all analyses.

Ascertainment correction of the family samples. To estimate the parameters of the regressive model (specifying the gene effect, residual familial dependencies, effects of risk factors, and variation of the hazard function with age), we needed to take into account the mode of ascertainment of the families. In the families unselected by family history, each family was ascertainment through one proband without any a priori knowledge of the family history of melanoma. The likelihood of the observations (i.e., melanoma status, ages, and risk factors categories) was corrected for the ascertainment bias using the approach proposed by Elston and Sobel (33). The probability that a family contains at least one proband is expressed in terms of the ascertainment probability π (probability for an affected offspring to become a proband), which was set to 0.01 for each family, because each family was selected through one case patient.

Each of the melanoma-prone families was selected because it included a large number of melanoma case patients and had at least two melanoma case patients with DNA available for CDKN2A typing. The mode of ascertainment of the melanoma-prone families was too complex to enable us to use the above procedure. However, to overcome the uncertainty of the precise reason for the selection of a specific family, one strategy is to calculate the likelihood of the observed phenotypes (melanoma status, ages, risk factors categories) and genotypes (CDKN2A mutation status), conditional on the observed phenotypes in all family members. This conditional likelihood represents an assumption-free method of ascertainment and leads to unbiased parameter estimates (34), although it may cause a substantial loss of the information contained in the observed data, from which the unknown parameters of the regressive model are estimated. This approach assumes that the disease-causing gene and marker (i.e., CDKN2A mutation) are tightly linked but are not in linkage disequilibrium (i.e., the two loci are not con-founded). Indeed, assuming that the CDKN2A locus is the disease-causing gene in the regressive model would remove most of the information available from the observed data and would hamper the estimation of the parameters of the regressive model. An alternative strategy is to calculate the joint likelihood of the observed phenotypes (melanoma status, ages, risk factors categories) and genotypes (CDKN2A mutation status) without correcting for ascertainment but assuming that CDKN2A is the disease-causing gene. Although use of the joint likelihood method may affect the estimates of the genetic parameters when the disease-causing gene is unknown, such an effect is less likely when the gene is known in most individuals (as in our data). Indeed, simulations (35) have shown that, in the situation in which the disease-causing gene is known, the joint likelihood approach was robust to correctly detect genetic and risk factor effects. Therefore, analysis of the melanoma-prone families was carried out using both the conditional and joint likelihood methods.

Estimation of parameters and tests of hypothesis. Parameter estimation and tests of models were carried out using maximum likelihood methods, as implemented in the computer program REGRESS (36), which performs such likelihood computations for family data. Nested models were compared by using likelihood-ratio tests. In segregation analysis, evidence for the effect of a major gene on melanoma risk was given by testing a model with no major gene and family dependence only against a model including both a major gene and family dependence. To support the presence of a major gene, Mendelian transmission of the putative major gene influencing melanoma risk should fit when compared with the general transmission model (30).

Three melanoma risk factors were included in the segregation analysis model: total nevi, sun exposure, and sunburn. We checked the statistical significance of these factors to melanoma risk by testing alternately the effect of each one (regression coefficient β set to zero versus β estimated) with all other genetic and risk factors included in the logistic regression models. Interactions between a putative major gene and these risk factors were tested by comparison of logistic regressive models in which the regression coefficients of risk factors, β's, were set equal to the same estimate of β, whatever g (no interaction), versus models in which three β's (or two β's under a dominant or recessive major gene model) were estimated (interaction). From the estimates of the β's coefficients of risk factors, the odds ratio of the hazard function associated with each risk factor [OR = \exp(\beta)] and 95% confidence interval (CI) were calculated.

In the combined segregation–linkage analyses of the melanoma-prone families, the effect of CDKN2A gene on melanoma risk was first tested by comparing a model with no effect of CDKN2A and family dependence only with a model including the effect of CDKN2A (a dominant mode of inheritance for CDKN2A was assumed) and family dependence. The effects of the risk factors on melanoma risk were tested by comparing a model without the effect of the tested risk factor (regression coefficient β for this factor set to zero) with a model including...
this factor (regression coefficient \( \beta \) for this factor estimated) while taking into account in both models the effect of CDKN2A and family dependence (and eventually other risk factors already included in the regressive model). Statistically significant risk factors were successively entered in the regressive model using a stepwise procedure. Gene–risk factor interactions were not tested in the melanoma-prone families.

**RESULTS**

**Descriptive Characteristics of the Family Samples**

Characteristics of the case patients and unaffected individuals in the unselected by family history and melanoma-prone families are shown in Table 1. The unselected by family history families (n=295) included 307 histologically confirmed cases of melanoma and 862 unaffected first-degree relatives. The mean (standard deviation (SD)) age at diagnosis of melanoma case patients was 45.7 ± 14.1 years. The melanoma-prone families (n=53) were mostly multigenerational and included 134 histologically confirmed melanoma cases and 478 unaffected first-, second-, and third-degree relatives. The two family samples had eight families in common but these eight families had different family structures in the two samples (i.e., nuclear families only in the families unselected by family history families and larger pedigrees, with additional affected and unaffected family members, in the melanoma-prone families).

Twenty of the melanoma-prone families had germline CDKN2A mutations that cosegregated with melanoma (Table 2), whereas the remaining 33 families were negative for both CDKN2A and CDK4 mutations (unpublished data). Among the melanoma-prone families, which all included at least two melanoma cases, the proportion of families with three or more melanoma cases was statistically significantly higher in the subset of families with CDKN2A mutations (65%) than in the subset of families without CDKN2A mutations (18%) (difference in proportion = 47%; \( P < .001 \)). In addition, the mean (SD) age at diagnosis of melanoma was statistically significantly younger in the subset of melanoma-prone families with CDKN2A mutations (39.7 ± 13.0 years) than in the subset without CDKN2A mutations (52.1 ± 15.4 years) (difference = 12.4 ± 2.5 years; \( P < .001 \)).

**Segregation Analysis of the Families Unselected by Family History**

Segregation analysis of melanoma in the families unselected by family history showed strong evidence for a dominant genetic effect on melanoma risk (\( P < .001 \)) and residual sib–sib dependence (\( P = .01 \)). Mendelian transmission of this major gene was not rejected when compared with the general transmission probability model (\( P = .22 \)).

Estimates of the effects and interactions of this major gene and risk factors for melanoma are presented in Table 3. The estimate of the deleterious allele frequency converged to a very small value and was fixed at .001 in all regression models. There was a clear variation of melanoma risk with age, which differed statistically significantly between genetically susceptible and nonsusceptible individuals (\( \delta_{AA} = 2.85 \) and \( \delta_{AA} = 5.42, P = .04 \)). Melanoma risk also varied with sex; the hazard rate in females was 1.67 times that in males (\( P < .001 \)). In addition, each of the other risk factors (total nevi, history of sun exposure, and

**Table 1. Characteristics of melanoma case patients and unaffected individuals from the unselected by family history and melanoma-prone families**

<table>
<thead>
<tr>
<th>Type of family</th>
<th>Melanoma case patients</th>
<th>Unaffected relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of melanoma case patients</td>
<td>Mean age at diagnosis, y (SD)</td>
</tr>
<tr>
<td>Unselected by family history (295 families)</td>
<td>307</td>
<td>45.7 (14.1)</td>
</tr>
<tr>
<td>Melanoma-prone family</td>
<td>73</td>
<td>52.1 (15.4)</td>
</tr>
<tr>
<td>without CDKN2A mutations (33 families)</td>
<td>61</td>
<td>39.7 (13.0)</td>
</tr>
<tr>
<td>with CDKN2A mutations (20 families)</td>
<td>64</td>
<td>55.0 (14.6)</td>
</tr>
</tbody>
</table>

*SD = standard deviation; — = no subject was tested for CDKN2A mutation in the unselected by family history families.
†Melanoma case patients include the probands and their affected relatives.
‡When no mutation was found in case patients, all unaffected individuals were considered as CDKN2A noncarriers.
Table 3. Effects and interactions of a major gene predisposing to melanoma and risk factors (i.e., total nevi, sun exposure, sunburn) by using the logistic regressive models that include a variation of melanoma risk with age and sex in 295 unselected by family history families*

<table>
<thead>
<tr>
<th>Model</th>
<th>Allele frequency</th>
<th>$\alpha_{aa}$</th>
<th>$\alpha_{Aa}$</th>
<th>$\delta$</th>
<th>$\beta_{\text{gender}}$</th>
<th>$\beta_{\text{total nevi}}$</th>
<th>$\beta_{\text{sun exposure}}$</th>
<th>$\beta_{\text{sunburns}**}$</th>
<th>Test statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Major gene and covariates, no interaction††</td>
<td>(0.0001)$\dagger\dagger$</td>
<td>-30.68</td>
<td>-15.75</td>
<td>5.42/2.85</td>
<td>(1.67)$§§$</td>
<td>1.71</td>
<td>(5.35)$§§$</td>
<td>(5.64)$§§$</td>
<td>(2.36)$§§$</td>
</tr>
<tr>
<td>2. Major gene $\times$ total nevi</td>
<td>(0.0001)</td>
<td>-30.75</td>
<td>-16.12</td>
<td>5.41/2.72</td>
<td>(1.73)$§§$</td>
<td>1.71/2.42$</td>
<td></td>
<td>$</td>
<td>1.71</td>
</tr>
<tr>
<td>3. Major gene $\times$ sun exposure</td>
<td>(0.0001)</td>
<td>-30.73</td>
<td>-16.96</td>
<td>5.44/3.18</td>
<td>(1.67)$§§$</td>
<td>1.76</td>
<td>(5.53)$§§$</td>
<td>(5.53)$§§$</td>
<td>(2.27)$§§$</td>
</tr>
<tr>
<td>4. Major gene $\times$ sunburns</td>
<td>(0.0001)</td>
<td>-30.76</td>
<td>-18.02</td>
<td>5.48/3.05</td>
<td>(1.68)$§§$</td>
<td>1.76</td>
<td>(5.37)$§§$</td>
<td>(5.37)$§§$</td>
<td>(2.16)$§§$</td>
</tr>
</tbody>
</table>

*Tests are based on likelihood ratio comparisons and are two-sided.
†Genotype-specific baseline parameter for noncarriers of the deleterious allele ($\alpha_{aa}$).
‡Genotype-specific baseline parameter for carriers of the deleterious allele ($\alpha_{Aa}$).
§§ is the regression coefficient specifying the variation of the hazard function with time (on the logarithmic scale) for noncarriers (aa) and carriers (Aa) of the deleterious allele.
$\beta_{\text{gender}}$ is the regression coefficient for females compared with males (referent group).
$\beta_{\text{total nevi}}$ is the regression coefficient for a binary variable indicating whether a subject has more than 50 nevi or less than 50 nevi (referent group).
$\beta_{\text{sun exposure}}$ is the regression coefficient for a binary variable indicating whether a subject was highly exposed to sun or not (referent group).
$\beta_{\text{sunburns}}$ is the regression coefficient for a binary variable indicating whether a subject had a history of sunburns or not (referent group).
††The model includes also sib–sib dependence (parameters not shown).
‡‡The allele frequency is fixed at 0.0001.
§§§The odds-ratios of the hazard function for each of the risk factors (OR = $\exp[\beta]$) are shown in parentheses below the regression coefficients.
¶¶Regression coefficients of the covariate are estimated in noncarriers (aa) and in carriers (Aa) of the deleterious allele.
$¶¶¶$Likelihood-ratio test of no interaction of a given covariate with the major gene (model 1) versus a model including that interaction (model 2, 3, or 4).

Combined Segregation–Linkage Analysis of the Melanoma-Prone Families

Combined segregation–linkage analysis of melanoma and CDKN2A mutation status in the melanoma-prone families, using the conditional likelihood strategy, led to unstable parameter estimates, so this conditional strategy was abandoned. Preliminary analyses using the joint likelihood strategy confirmed the statistically significant association (P < .001) between CDKN2A mutations and melanoma risk. The variation of the effect of CDKN2A (presence/absence of mutation) on melanoma risk with age did not differ between mutation carriers and noncarriers or between males and females (data not shown), as also observed in the European families in the Melanoma Genetics Consortium (21). To test for the effects of other factors influencing melanoma risk (e.g., pigmentary traits, nevus phenotypes, sun exposure, skin reactions to sunlight), we set the parameters specifying the time-dependent association between CDKN2A mutations ($\alpha$, and $\delta$ parameters) and melanoma risk to the estimates that were obtained from the European families in the Melanoma Genetics Consortium study, by using the conditional likelihood approach (21) because this approach is known to be less prone to bias on these genetic parameters than the joint likelihood strategy (34).

Tests for statistically significant risk factors for melanoma, using a stepwise logistic regression procedure, are shown in Table 4. The statistically significant factors were dysplastic nevi (P < .001), sunburn (P < .001), and total nevi (P = .009). The next risk factor that entered the regression model was sun exposure, which did not reach statistical significance (P = .06). The odds ratio of the hazard function of melanoma associated with dysplastic nevi was 2.32 (95% CI = 2.08 to 2.58), whereas the odds ratio of the hazard function of melanoma associated with sun exposure reached 0.09 and 0.15 by age 50 years and 0.49 and 0.51 by age 80 years in males and females, respectively.

The estimates of the age-specific risk associated with the major gene according to the genotype at the major locus and sex are shown in Fig. 1. In carriers of the melanoma-predisposing gene, this risk of melanoma reached 0.09 and 0.15 by age 50 years and 0.49 and 0.67 by age 80 years in males and females, respectively (Fig. 1, A). Interestingly, these risk estimates were close to the melanoma risk estimate associated with CDKN2A mutations found in the 31 European melanoma-prone families included in the Melanoma Genetics Consortium study (21) in which this risk reached 0.13 by age 50 years and 0.58 by age 80 years (Fig. 1, A). In noncarriers of the melanoma-predisposing gene (Fig. 1, B), the estimated age-specific risk reached 0.009 and 0.015 by age 80 years in males and females, respectively. These risk estimates were close to those obtained in noncarriers of CDKN2A mutations of the European families in the Melanoma Genetics Consortium study (21) and were also similar to the age-specific cumulative incidence of melanoma estimated from the French Tumor Registry data (38).
Analysis of the subset of melanoma-prone families with CDKN2A mutations showed that dysplastic nevi and sunburn remained statistically significant risk factors for melanoma (dysplastic nevi, OR = 4.57, 95% CI = 3.62 to 5.78, and sunburn, OR = 4.53, 95% CI = 3.66 to 5.59; both P < .001), whereas total nevi was no longer a statistically significant risk factor for melanoma (total nevi, OR = 2.23, 95% CI = 1.10 to 4.42; P = .09 from a likelihood-ratio test with 2 degrees of freedom for testing the effect of total nevi using the missing indicator method).

Among the host factors and sun-related covariates that enhanced melanoma risk in addition to the melanoma-predisposing gene in unselected families (or CDKN2A in melanoma-prone families), total nevi and sunburn were statistically significant associated with melanoma risk in both the unselected by family history and melanoma-prone families. The age-specific risks of melanoma associated with high numbers of nevi and sunburn in genetically susceptible individuals are shown in Fig. 2. The estimated melanoma risk associated with high nevus count was lower in the melanoma-prone families than in the unselected by family history families (Fig. 2, A). For example, by 50 years of age, this risk was 0.25 (95% CI = 0.23 to 0.27) in individuals from the melanoma-prone families and 0.41 (95% CI = 0.36 to 0.47) and 0.58 (95% CI = 0.52 to 0.65) in males and females, respectively, from the unselected by family history families. In addition, whereas the lifetime risk (i.e., by age 80 years) reached 0.82 (95% CI = 0.79 to 0.85) in individuals from the melanoma-prone families, it reached 0.97 (95% CI = 0.95 to 0.98) and 0.99 (95% CI = 0.98 to 1.00) in males and females, respectively, from the unselected by family history families. Interestingly, the point estimates of melanoma risk in gene carriers with sunburn were similar in the two family types (Fig. 2, B), reaching 0.52 (95% CI = 0.49 to 0.54) by 50 years of age in individuals from the melanoma-prone families and 0.42 (95% CI = 0.14 to 0.85) and 0.59 (95% CI = 0.22 to 0.96) in males and females, respectively, from the families unselected by family history. The estimate for melanoma risk was higher than 0.98 for all individuals by age 80 years.

**DISCUSSION**

Epidemiologic studies have shown that clinical, environmental, and genetic factors influence the risk of melanoma. The two main issues addressed by this study were 1) the comparison of the association of the melanoma-predisposing gene with melanoma risk in families unselected by family history with the association of the CDKN2A gene with melanoma risk in melanoma-prone families and 2) the influence of other risk factors (e.g., pigmentary traits, nevus phenotypes, history of sun exposure, skin reactions to sunlight) on melanoma risk in the two family types. Segregation analysis of melanoma in the 295 unselected families showed evidence for the transmission of a dominant major gene and residual sib–sib dependence. We found that the penetrance of this major gene was of the same order of magnitude as that of the CDKN2A gene estimated from the 31 European melanoma-prone families of the Melanoma Genetics Consortium (21). Although the parameter estimates specifying the age-dependent association between the major gene and melanoma risk (αg and δg parameters) seemed to differ between the families unselected by family history (Table 3) and the European melanoma-prone families in the Melanoma Genetics Consortium (Table 4), they led to similar age-specific melanoma risks, which are expressed only in terms of these parameters but depend also on the range of the observed age-values (starting at 5 years of age in the families unselected by family history and at 15 years of age in the melanoma-prone families).

In the families unselected by family history, the penetrance of the melanoma-predisposing gene, whose identity is not yet known but which may be acting as CDKN2A, was enhanced by three risk factors: total nevi, sun exposure, and sunburn. Two of these risk factors, total nevi and sunburn, were statistically significantly associated with melanoma risk in the melanoma-prone families, whereas sun exposure was not. The additional association of dysplastic nevi with melanoma risk found in the melanoma-prone families was not tested in the unselected by family history families because of missing data. Excluding dysplastic nevi from the analysis of the melanoma-prone families led to statistically significant evidence for the effects of three risk factors (total nevi, sun exposure, sunburn), in addition to CDKN2A mutations, on melanoma risk; these risk factors were
of a melanoma predisposing gene estimated from the gene and risk factors (added by stepwise procedure) in 53 melanoma-prone families and the subset of families with CDKN2A mutations*

<table>
<thead>
<tr>
<th>Model</th>
<th>Allele frequency CDKN2A</th>
<th>$\alpha_{\text{Aa}}$†</th>
<th>$\alpha_{\text{AA}}$†</th>
<th>$\delta$</th>
<th>$\beta_{\text{dysplastic nevi}}$</th>
<th>$\beta_{\text{sunburns}}$</th>
<th>$\beta_{\text{total nevi}}$#</th>
<th>$\beta_{\text{sun exposure}}$$^*$</th>
<th>Test statistic</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. No risk factor</td>
<td>(0.0001)††</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>2.27 (9.68)††</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1b. No risk factor</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>1.07 (2.92)††</td>
<td>1.83 (6.23)††</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>96.75§§ &lt;.001</td>
<td></td>
</tr>
<tr>
<td>2a. Adding dysplastic nevi to model 1a</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>0.84 (2.32)††</td>
<td>1.64 (5.16)††</td>
<td>0.69 (1.99)††</td>
<td>—</td>
<td>—</td>
<td>9.49 &lt;.009</td>
<td></td>
</tr>
<tr>
<td>2b. Adding dysplastic nevi to model 1b</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>0.83 (2.29)††</td>
<td>1.51 (4.53)††</td>
<td>0.67 (1.95)††</td>
<td>0.49 (1.63)††</td>
<td>—</td>
<td>5.52 &lt;.001</td>
<td></td>
</tr>
<tr>
<td>3a. Adding sunburns to model 2a</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>1.30 (3.67)††</td>
<td>1.26 (3.53)††</td>
<td>0.80 (2.23)††</td>
<td>—</td>
<td>—</td>
<td>4.73 &lt;.001</td>
<td></td>
</tr>
<tr>
<td>3b. Adding sunburns to model 2b</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>2.91 (18.36)††</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>85.93 &lt;.001</td>
<td></td>
</tr>
<tr>
<td>4a. Adding total nevi to model 3a</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>1.26 (3.53)††</td>
<td>0.83 (2.29)††</td>
<td>1.51 (4.53)††</td>
<td>0.67 (1.95)††</td>
<td>0.49 (1.63)††</td>
<td>—</td>
<td>5.52 &lt;.001</td>
</tr>
<tr>
<td>4b. Adding total nevi to model 3b</td>
<td>(0.0001)</td>
<td>(−16.11)</td>
<td>(−11.67)</td>
<td>(2.01)</td>
<td>0.67 (1.95)††</td>
<td>0.83 (2.29)††</td>
<td>1.51 (4.53)††</td>
<td>0.67 (1.95)††</td>
<td>0.49 (1.63)††</td>
<td>—</td>
<td>5.52 &lt;.001</td>
</tr>
<tr>
<td>Subset of families with CDKN2A mutations (N = 20)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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<td>—</td>
</tr>
</tbody>
</table>

*Tests are based on likelihood ratio comparisons and are two-sided. — = the corresponding risk factor was not included in the regressive model.
†Genotype-specific baseline parameter for noncarriers of CDKN2A mutation ($\alpha_{\text{Aa}}$) is fixed at the value estimated in the European families of the Melanoma Genetics Consortium (21).
† Genotype-specific baseline parameter for carriers of CDKN2A mutation ($\alpha_{\text{AA}}$) is fixed at the value estimated in the European families of the Melanoma Genetics Consortium (21).
§§ Regression coefficient specifying the variation of the hazard function with time (on the logarithmic scale) is fixed at the value estimated in the European families of the Melanoma Genetics Consortium (21). Note that the penetrance of CDKN2A estimated from the $\alpha_{\text{Aa}}$, $\alpha_{\text{AA}}$, and $\delta$ values shown in this table was similar to that of a melanoma predisposing gene estimated from the $\alpha_{\text{Aa}}$, $\alpha_{\text{AA}}$, and $\delta$ parameters shown in Table 3 but using different ages of age values.
‡‡ Regression coefficient for a binary variable indicating whether a subject has dysplastic nevi or not (referent group).
§§ Regression coefficient for a binary variable indicating whether a subject has sunburns or not (referent group).
** Regression coefficient for a binary variable indicating whether a subject is highly exposed to sun or not (referent group).
†† The allele frequency is fixed at 0.0001 (see “Methods”).
†† The odds-ratios of the hazard function for each of the risk factors (OR = exp[$\beta$]) are shown in parentheses.
§§ Likelihood ratio test of the absence of a given risk factor versus a model including the risk factor (the number of degrees of freedom is 2 because each risk factor is split into a missing indicator variable [not shown] and an exposure variable). Risk factors are successively incorporated in the model by a stepwise procedure.

the same as those found in the families unselected by family history (results not shown). To our knowledge, this study is first to show that genetic factors, nevus phenotypes, and sun-related covariates jointly influence melanoma risk in both families unselected by family history and melanoma-prone families.

Our results are in agreement with the Melanoma Genetics Consortium, which found that variation in melanoma risk according to geographic location is similar in CDKN2A mutation carriers and noncarriers, suggesting that the same factors that mediate population incidence of melanoma may also mediate CDKN2A penetrance (21). However, our results differ from those reported from family studies (23, 24) of breast cancer, in which the breast cancer risk attributed to BRCA1 and BRCA2 mutations estimated from high-risk families was much higher than estimated from families ascertained through population-based incident cases of cancer. Further determination of the presence of CDKN2A mutations in population-based samples will allow a direct comparison of the penetrance of this gene in families ascertained through incident cases of melanoma with that obtained in high-risk families.

The outcomes of our segregation analysis differ from a similar analysis of Australian population-based families (5), in which there was strong statistical evidence for familial dependence in melanoma occurrence but no clear identification of a major gene. However, the Australian study examined only the familial transmission of melanoma without taking into account potential risk factors. The results from the melanoma-prone families in this analysis agree partly with those of a combined segregation–linkage analysis of 20 American melanoma-prone families genotyped for CDKN2A (20), in which presence of dysplastic nevi and numbers of total nevi were found to increase melanoma risk in addition to the CDKN2A gene, whereas solar injury did not have a statistically significant effect on risk. Moreover, the increase in melanoma risk associated with dysplastic nevi was statistically significantly higher in individuals without CDKN2A mutations than in those with mutations. We did not test for interactions between the CDKN2A gene and risk factors in our high-risk families (i.e., the melanoma-prone families) because such a test would represent a test for heterogeneity rather than an actual test for interaction. Indeed, all but two of the melanoma case patients who did not carry a CDKN2A gene mutation belonged to the 33 melanoma-prone families without CDKN2A mutations, and the development of melanoma in these patients may depend on yet-unknown and possibly different genetic factors.

Epidemiologic studies have demonstrated that nevus phenotypes, both numbers of nevi and presence of dysplastic nevi, are major risk factors for melanoma (16). When these two nevus phenotypes were considered together in the melanoma-prone families, they both influenced melanoma risk, with dysplastic
In our study with CDKN2A mutations in many families, including those in melanoma-prone families, there was statistically significant evidence for familial aggregation of the phenotype defined by a high number of nevi, this familial clustering decreasing with the number of melanoma cases in the families, suggesting that both common and different genetic mechanisms may be involved in the development of a high number of nevi and melanoma (45). Thus, selecting families by family history of melanoma may lead to selecting preferentially the factors underlying melanoma rather than those underlying a high number of nevi. Consequently, the total nevi risk factor would play a less important role in melanoma risk in melanoma-prone families than in families unselected by family history. Therefore, the genetic basis of nevi development and their association with melanoma development needs to be further investigated.

Sun exposure is the major environmental risk factor associated with melanoma, with the increased risk due mainly to intermittent exposure (15). A history of sunburn, which is an indicator of intermittent sun exposure but is also related to skin type, has also been reported by many epidemiologic studies to be associated with melanoma (15). We found that both sun exposure and a history of sunburn influenced melanoma risk in the families unselected by family history, whereas only a history of sunburn was associated with melanoma risk in the melanoma-prone families. This finding for sun exposure is in agreement with a recent Australian population-based study, which compared melanoma patients and their unaffected relatives from families at high, intermediate, and low familial melanoma risk and found a statistically significant association of sun exposure with melanoma risk in the subjects from families at low or intermediate familial melanoma risk but not in the subjects from families at high familial melanoma risk (46). The point estimate of the odds ratio for sun exposure (OR = 5.37) associated with increased melanoma risk in our families unselected by family history is similar to the upper range of odds ratios estimated in published case–control studies, especially in those from France, Spain, and Germany (15). The melanoma risk associated with sunburn was higher in genetically susceptible individuals than in nonsusceptible individuals in the families unselected by family history and was similar in the melanoma-predisposing gene carriers from the families unselected by family history and in the CDKN2A mutation carriers from the melanoma-prone families. This finding suggests common mechanisms and/or interactions between the p16 pathway and the UV-sensitivity pathway.

Dysplastic nevi did not appear to cosegregate with CDKN2A mutations in many families, although alterations in the p16 pathway may increase melanocyte proliferation (39), several studies have suggested that nevus phenotypes may result from different genetic mechanisms (40). Dysplastic nevi did not appear to cosegregate with CDKN2A mutations in many families, including those in our study (9,41–43) and the presence of dysplastic nevi has been found to be an important predictor of melanoma risk in melanoma-prone families, regardless of CDKN2A mutation status (44). In 66 French families, who had at least two melanoma cases per family, there was statistically significant evidence for familial aggregation of the phenotype defined by a high number of nevi, this familial clustering decreasing with the number of melanoma cases in the families, suggesting that both common and different genetic mechanisms may be involved in the development of a high number of nevi and melanoma (45). Thus, nevi conferring a higher risk than total nevi, which was similar to the study in 20 American families (20). The increase in melanoma risk associated with total nevi was higher in the unselected by family history families than in the melanoma-prone families. Although alterations in the p16 pathway may increase melanocyte proliferation (39), several studies have suggested that nevus phenotypes may result from different genetic mechanisms (40). Dysplastic nevi did not appear to cosegregate with CDKN2A mutations in many families, including those in our study (9,41–43) and the presence of dysplastic nevi has been found to be an important predictor of melanoma risk in melanoma-prone families, regardless of CDKN2A mutation status (44). In 66 French families, who had at least two melanoma cases per family, there was statistically significant evidence for familial aggregation of the phenotype defined by a high number of nevi, this familial clustering decreasing with the number of melanoma cases in the families, suggesting that both common and different genetic mechanisms may be involved in the development of a high number of nevi and melanoma (45). Thus, selecting families by family history of melanoma may lead to selecting preferentially the factors underlying melanoma rather than those underlying a high number of nevi. Consequently, the total nevi risk factor would play a less important role in melanoma risk in melanoma-prone families than in families unselected by family history. Therefore, the genetic basis of nevi development and their association with melanoma development needs to be further investigated.

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family samples from different geographical areas may be of interest to compare the effects of melanoma risk factors in populations with different incidence rates of melanoma. Determination of CDKN2A mutation status in population-based samples, taking into account the effect of other genes, such as MC1R, as well as environmental, lifestyle, and other potential risk factors may also prove to be a fruitful area of investigation in terms of assessing the respective roles of genetic and nongenetic determinants in the complex etiology of melanoma. Such studies may have important consequences to improve the prediction of melanoma risk at the population level and in different familial settings and to better define effective prevention and surveillance strategies of this cancer.

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NOTES

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