Melanocortin-1 Receptor (MC1R) Gene Variants and Dysplastic Nevi Modify Penetrance of CDKN2A Mutations in French Melanoma-Prone Pedigrees

Valérie Chaudru,1 Karine Laud,2 Marie-Françoise Avril,2 Annie Miniere,2 Agnès Chompret,2 Brigitte Bressac-de Paillerets,2 Florence Demenais,3 and The French Familial Melanoma Study Group

1Institut National de la Santé et Recherche Médicale and Université d’Evry, Evry, France and 2Institut Gustave Roussy, Villejuif, France

Abstract

Germline mutations in CDKN2A gene predispose to melanoma with high but incomplete penetrance. Penetrance of CDKN2A gene was found to be significantly influenced by host factors (nevus phenotypes and sunburn) on one hand and by variants of MC1R gene (RHC variants consistently associated with red hair and fair skin) on the other hand. Our goal was to examine the joint effects of MC1R variants and other potential risk factors (total nevi, dysplastic nevi, pigmentary traits (skin, hair and eye color), skin reactions to sunlight, and degree of sun exposure) on CDKN2A penetrance. Clinical, genetic, and covariate data were recorded in 20 French melanoma-prone families with cosegregating CDKN2A mutations. Analysis of the cotransmission of melanoma and CDKN2A mutations was conducted by likelihood-based methods using the regressive logistic models, which can account for a variation of disease risk with age and can include the aforementioned risk factors as covariates. RHC variants, considered either alone or in the presence of pigmentation and nevus phenotypes, were found to increase significantly CDKN2A penetrance. Multivariate analysis, using a stepwise selection procedure, showed significant effects of two factors on melanoma risk in CDKN2A mutations carriers: RHC variants [odds ratio of hazard function (OR), 2.21; P = 0.03] and dysplastic nevi (OR, 2.93; P < 0.01). Such results may have important consequences to improve the prediction of melanoma risk in families.

(Cancer Epidemiol Biomarkers Prev 2005;14(10):2384–90)

Introduction

Cutaneous malignant melanoma (CMM) is a complex disease resulting from genetic and other risk factors. Approximately 10% of malignant melanomas are observed in individuals with a family history. Epidemiologic studies have shown that exposure to sunlight is the major environmental risk factor associated with melanoma, whereas high numbers of melanocytic nevi, both clinically banal and atypical (dysplastic), hair color, eye color, skin color, extent of freckling, and skin reactions to sun exposure are the major host factors (see ref. 1, for a review).

Two melanoma high-risk genes have been identified in melanoma-prone families: the cyclin-dependent kinase inhibitor 2A (CDKN2A; refs. 2, 3) that encodes two distinct proteins translated in alternative reading frames, p16INK4A and p14ARF; and the cyclin-dependent kinase 4 (CDK4; ref. 4). The p14ARF and CDK4 proteins are implicated in the same cell cycle pathway, pRB-regulated G1-S phase transition (5, 6), whereas the p14ARF protein acts by the p53 pathway to induce cell cycle or apoptosis (7). Most germline CDKN2A mutations affect the p16INK4A protein and such mutations have been observed in ~20% (range, 5% to <50%) of tested melanoma-prone families from Australia, Europe, and North America (8, 9). CDK4 acts as an oncogene and germline mutations of this gene have been found in only three melanoma-prone families worldwide (4, 8), whereas both CDK4 and CDKN2A mutations have been found to segregate in one Australian kindred (10).

Besides these two high-penetrance genes, common genetic variants with a more modest effect on melanoma risk have been reported such as those encoding proteins involved in pigmentation, DNA repair, cell growth, and differentiation or detoxification of metabolites (see ref. 11, for a review). One of the most studied is the melanocortin-1 receptor gene (MC1R), which has been shown to play a crucial role in human pigmentation (12). Moreover, some variants of this gene were found to increase melanoma risk (13, 14). MC1R is a seven-pass transmembrane G-protein-coupled receptor for α-melanocyte-stimulating hormone (α-MSH) and is expressed on many cell types, including melanocytes (15). Binding of α-MSH to MC1R normally activates adenyl cyclase and increases intracellular cyclic AMP production, which leads to a switch in melanin production from red/yellow pheomelanin pigments to brown/black eumelanosins (16). Three variants, R151C, R160W, and D294H (also called RHC variants), were found to be consistently associated with fair skin and red hair (see ref. 16, for a review). RHC and other variants also increased risk for ephelides or solar lentigines (17). Case-control studies in different populations reported that RHC and other variants were associated with melanoma risk in individuals with dark skin (13) or independently of skin type (14) and, more recently, independently of UV exposure and clinical risk factors (skin, hair and eye color, solar lentigines, and nevus count; ref. 18). This suggests that MC1R gene may play a role in melanoma pathogenesis not only through its effect on melanin synthesis but also through a more direct effect on melanocytic cellular transformation.

Received 10/24/04; revised 7/28/05; accepted 8/12/05.

Grant support: Fondation de France grants 007051 and HDH01E, Programme Hospitalier de Recherche Clinique Regional 2001 Be-de-France grant AOR 01 091, National Cancer Institute grant RO1 CA 063115, Institut Gustave Roussy postdoctoral fellowship grant CRC 2001 (K. Laud), Conseil Régional d’Île-de-France grant H01H1G (computer facilities), Institut National de la Santé et de la Recherche Medicale, Université d’Evry, and Genopole.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertised in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

Note: V. Chaudru and K. Laud contributed equally to this study.

The French familial melanoma study group members are listed in the Acknowledgments.

Requests for reprints: Valérie Chaudru, Institut National de la Santé et de la Recherche Medicale, Université d’Evry, EMB 8006, Tour Evry 2, 523 Place des Terrasses de l’Agora, 91034 Evry, France. Phone: 33-1-6057-3850; Fax: 33-1-6057-3848. E-mail: chaudru@evry.inserm.fr

Copyright © 2005 American Association for Cancer Research.

do:10.1158/1055-9965.EPI-04-0777

Cancer Epidemiol Biomarkers Prev 2005;14(10):2384–90

Downloaded from cebp.aacrjournals.org on June 24, 2017. © 2005 American Association for Cancer Research.
Although CDKN2A mutations confer a high risk of melanoma, penetrance of this gene is incomplete. Examination of the penetrance of CDKN2A mutations in 80 multiple-case families from Europe, Australia, and the United States, as part of the Melanoma Genetics Consortium, has indicated a variation of this penetrance according to geographic location (19). By age 80 years, CDKN2A mutation penetrance reached 58% in Europe, 76% in the United States, and 91% in Australia. A few host factors have been shown to enhance significantly CDKN2A penetrance in families with cosegregating CDKN2A mutations: total nevi and dysplastic nevi in 13 North American pedigrees (20) and dysplastic nevi and sunburn in 20 French pedigrees (21). Moreover, MC1R variants were found to increase penetrance of mutations in CDKN2A (22, 23). This increase was mainly due to RHC variants in 15 Australian pedigrees (22) and to R151C variant in six Dutch families (23). However, these two latter studies did not take into account the effects of other melanoma risk factors, such as nevus phenotypes, pigmentedary traits, or sun-related covariates, except for skin color in the Dutch study (23).

The goal of the present study was to examine the joint effects of MC1R variants, nevus phenotypes, pigmentary traits, history of sun exposure, and skin reactions to sunlight on CDKN2A penetrance using a different analytic approach from the ones previously used (22, 23). Analysis of the cotransmission of CMM and CDKN2A mutations in 20 French melanoma-prone families was conducted by a likelihood-based method using regressive logistic models. These models can include the effects of other melanoma risk factors, such as nevus phenotypes, pigmentedary traits, or sun-related covariates, except for skin color in the Dutch study (23).

Patients and Methods

Ascertainment of Families and Data Collection. The 20 melanoma-prone families available for the present study were identified as part of the French Familial Melanoma Project (as described in details in refs. 21, 27). In brief, a sample of 100 families with at least two melanoma cases has been recruited since 1986 from the Department of Dermatology at the Institut Gustave Roussy and other French hospitals forming the French Familial Melanoma Study Group. Eligible probands were defined as White subjects living in France for >10 years, who had a newly diagnosed and histologically confirmed melanoma. From the total sample of 100 families, 53 families were eligible for CDKN2A mutation testing, because they included at least two histologically confirmed melanoma cases with DNA available among first- or second-degree relatives. Genotyping for CDKN2A germline mutations has led to identification of 20 melanoma pedigrees who contained a total of 14 different CDKN2A mutations: L16P, 19ins6, G23N, R24P, M53I, S56I, V59G, L62P, del 67-71, A68L, N71K, G101W, FS102-119X, and V126D (21).

Family data were collected by interviewing the probands on their first-degree, second-degree, and third-degree relatives and included demographic characteristics (gender, date of birth, and if deceased, age at death and cause of death) and the occurrence of melanoma and any other cancer, along with the age at diagnosis. Confirmation of melanoma diagnosis was sought through medical records, review of pathologic material, and/or pathology reports. Only histologically confirmed melanomas were considered as affected in the analyses. Physical examination was conducted by a trained dermatologist in all probands and 70% of probands’ relatives. A questionnaire, recording data on various risk factors, was completed by the probands and relatives seen in the hospital and was distributed by probands to all other relatives. The data recorded on the questionnaire included skin color (pale or dark), eye color (pale or dark), hair color (red, blond, light brown, dark brown, or dark), number of nevi (<10, 10-50, >50 nevi), presence of atypical/dysplastic nevi (moles ≥5 mm in diameter with irregular margins and variegated color, only for those examined by a dermatologist), degree of exposure to sunlight (low, medium, or high) during holidays, leisure time and work time, artificial 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 before participation under an Institutional Review Board–approved protocol.

Genotyping of MC1R Variants. Genomic DNA was extracted from peripheral blood lymphocytes using the QiaAmp DNA blood midi kit (Qiagen, Chatsworth, CA). A specific amplification of MC1R-coding sequence was done with the following primers: forward, NT-F 5’-GACGCACT-GAACTAAGCA-3’ and reverse, NT-R 5’-CAGGTCACAG-GAAGCCCA-3’ (MWG, Ebersteg, Germany) as described by Kanefsky et al. (24). This primer set is located in the noncoding region enclosing the MC1R gene and resulting in a 1,125-bp PCR fragment. The PCR reactions were done in a final volume of 50 μL with 50 ng of genomic DNA, 200 μmol/L of each deoxynucleotide triphosphate (Pharmacia LKB Biotechnology, Orsay, France), 0.5 μmol/L of each primer, 1.25 IU Taq DNA polymerase Hot Start (Qiagen), and 1× PCR hot start buffer (Qiagen). The reaction of PCR was initially denatured for 10 minutes at 94°C followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 45 seconds at 72°C and a final extension step at 72°C for 10 minutes. Five microliters of PCR products were purified with ExoSAP-IT method (U.S. Biochemical, Cleveland, OH).

To identify MC1R variants, PCR products were sequenced with the same primers used for PCR and two additional internal primer sets. The sequence of the internal primers were TM-F, 5’-AACCTGCACCTACCCATGTA-3’; TM-R, 5’-TTTAAGGCACAAACGCTCTGTT-3’; CT-F, 5’-TCGCCTCATGGCAGCACCATC-3’; and CT-R, 5’-CAGGGTCACACAGGAAACC-3’.

The sequencing of MC1R was done with 2.5 μL of DNA template of PCR, 4 μL of Big Dye (Perkin-Elmer, Applied Biosystem, Foster City, CA), and 1.5 pmol of each primer. PCR products were purified by solid-phase extraction through sephacryl G50 and subsequently analyzed using an ABI 377 sequencer (Perkin-Elmer, Applied Biosystem).

Genotyping of MC1R variants was conducted in all members of 20 melanoma families with DNA available and in a sample of 172 controls. Controls, free of melanoma, belonged to breast and ovarian cancer families followed at Institut Gustave Roussy.

Statistical Analysis. Comparison of MC1R allele frequencies in controls and members of the melanoma-prone families was assessed by either a Fisher’s exact test or a Pearson’s χ² test, depending on the sample size.

Effects of MC1R variants and other potentially risk factors (e.g., nevus phenotypes, pigmentedary traits, history of sun exposure, and skin reactions to sunlight) on CDKN2A penetrance were estimated using combined segregation-linkage analysis. This analysis of the cotransmission of melanoma and CDKN2A mutations in the 20 melanoma-prone families was based on the regressive logistic models (24) extended to take into account a variable age at diagnosis of disease (26) and linked marker loci (25). 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 disease-causing locus (CDKN2A) multiplied by the penetrance function summed over individuals with an unknown CDKN2A genotype. The probability of unobserved
genotypes is expressed in terms of the frequency of CDKN2A mutations in the general population for those individuals who have no ancestors in the pedigrees (founders of the pedigrees and spouses) and in terms of Mendelian probabilities for those with ancestors in the pedigrees. The frequency of CDKN2A mutations in the population was assumed to be 0.0001, as in previous analyses (19, 21).

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

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

where \( g_i \) is the \( i \)th person's genotype at CDKN2A locus 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 (26). Because there was no subject affected with melanoma under 15 years of age in the melanoma-prone families, the period of follow-up was taken from 15 years of age to age at diagnosis for affected individuals, age at examination for unaffected subjects (or affected subjects with unknown age at diagnosis), or age at death for deceased subjects. The penetrance function is then derived from the hazard function \( \lambda(k) \), which is the probability of being affected in the \( k \)th interval given unaffected before. The hazard function \( \lambda(k) \), 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:

\[
\theta_i(k) = \beta g_i + \beta X_i + u_i(k)
\]

where \( g_i \) is the genotype-specific baseline parameter, \( \beta g \) is the row vector of genotype-specific regression coefficients for covariates \( X \), and \( u_i(k) \) is a function of \( k \) that represents the variation of the logit of the hazard function with time and can be genotype dependent. The hazard function can be assumed to be constant over time \( u_i(k) = 0 \) or varying with time using different parametric functions of \( k \). We found that the function \( u_i(k) = \delta g \cdot \ln(k) \) fit better the data than a polynomial function of \( k \) and was then used in all analyses.

All covariates were dichotomized with the baseline and at-risk categories being defined as follows: hair color (dark or dark brown versus light brown, blond, or red), skin color (dark versus pale), propensity to sunburn (light versus dark), skin color (dark versus light brown, blond, or red), and dysplastic nevi (absent versus present). For each MC1R variant, the baseline category included MC1R consensus homoygotes, whereas the at-risk category included subjects with one or two variant alleles. Only dominant effects were considered, because the frequency of homozygote subjects for each variant was <2%. The present analysis considered individually each MC1R variant with frequency ≥5% in the family sample as well as the pool of RHC variants (R151C, R160W, and D294H). To deal with missing covariates, we used the complete case method, which implies coding subjects with missing covariates as unknown for disease status (29).

Likelihood computation of a family sample usually involves correction for ascertainment of the families when they are not drawn at random from the population. Each of the melanoma-prone families was selected, because it included at least two melanoma cases with DNA available for CDKN2A genotyping and at least one of them was carrier of CDKN2A mutations. This mode of ascertainment was too complex to be corrected analytically. In such situation, one strategy is to calculate the likelihood of the observed phenotypes (melanoma status, age, and covariates) 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 when the ascertainment is based on phenotype (30). However, this approach may cause a substantial loss of the information and may hamper the estimation of the parameters of the regressive model, especially when covariates are included in the model. An alternative strategy is to calculate the joint likelihood of the observed phenotypes (melanoma status, age, and covariates) and genotypes (CDKN2A mutation status) without correcting for ascertainment but assuming that CDKN2A is the disease-causing gene. Although use of joint likelihood method may overestimate the parameters of the disease gene, tests of the effects of covariates are likely to be valid when the disease-causing gene is known and most subjects have been genotyped. Indeed, analysis of simulated data under different sampling schemes has shown that the joint likelihood approach was robust to noncorrection for ascertainment and detected the generated model in terms of genetic and risk factor effects as well as gene × risk factors interactions when the disease-causing gene was known (31). Provided that conditional likelihood led to numerical problems (unstable parameters estimates), we used the joint likelihood strategy, restricting our analyses to (a) CDKN2A mutation carriers and (b) all individuals genotyped for CDKN2A (all nongenotyped subjects being coded as unknown for melanoma status and thus bringing no contribution to the likelihood). Indeed, restricting the analyses to genotyped subjects without inferring CDKN2A mutation status in untyped subjects can minimize possible bias on parameter estimates.

Parameter estimation and tests of models were carried out using maximum likelihood methods, as implemented in the computer program REGRESS (32). Nested models were compared by use of likelihood ratio tests. Effects of the covariates (MC1R variants and other potential risk factors) on the penetrance function were tested by comparing a model without the effect of the tested covariate (regression coefficient \( \beta \) for this covariate set to zero) with a model including this covariate (regression coefficient \( \beta \) for this covariate estimated), whereas taking into account the effect of CDKN2A (and eventually other covariates already included in the regressive model). The odds ratio (OR) of hazard function associated with each covariate \( OR = \exp(\beta) \) and 95% confidence interval (95% CI) were computed from the corresponding estimate of the \( \beta \) parameter. Effect of each MC1R variant (or pooled RHC variants) on CDKN2A penetrance was first tested in univariate analysis. For each variant showing a significant effect, we tested alternatively whether that effect remained in the presence of each host factor which may be a potential confounder. Finally, effects of MC1R variants, host-related phenotypes, and degree of sun exposure were considered jointly in multivariate analysis and their significance was tested using a stepwise procedure.

Results

The 20 French melanoma-prone families (comprising a total of 326 subjects), in which 14 different CDKN2A mutations were segregating (21), included 61 melanoma cases (mean age at diagnosis of 39.70 ± 13.02 years), 214 unaffected subjects (mean age at examination of 46.0 ± 20.1 years), and 51 subjects with unknown affection status. CDKN2A mutation status was determined in 160 subjects (51% being mutation carriers). MC1R genotype was available for 159 family members (31% having one variant and 52% having more than one variant).

The distribution of a total of 153 subjects genotyped for both CDKN2A and MC1R according to melanoma status is shown in Table 1. Among the 44 melanoma cases, only two subjects were noncarriers of CDKN2A mutations; whereas among the
106 unaffected subjects, 37 subjects were carriers of CDKN2A mutations (three subjects had an unknown melanoma status). The frequency of subjects carrying none, one, two, or three MC1R variants was significantly different in affected and unaffected carriers of CDKN2A mutations (P = 0.001). Interestingly, in these CDKN2A mutation carriers, there was a higher number of unaffected than affected subjects with one MC1R variant, whereas the inverse trend was observed in subjects with at least two MC1R variants. Moreover, the mean age at diagnosis of melanoma in affected individuals and the mean age at examination in unaffected subjects did not differ according to the number of MC1R variants in both carriers and noncarriers of CDKN2A mutations (P > 0.40).

Allele frequencies of the 14 MC1R variants, detected in the present sample, are shown in Table 2. All MC1R variants were in Hardy-Weinberg equilibrium (P > 0.15). The allele frequencies were not significantly different between affected and unaffected members of the 20 melanoma-prone families when we considered all genotyped subjects or CDKN2A mutation carriers only (P > 0.06). When comparing 159 members of melanoma-prone families (pool of affected and unaffected) and 172 controls, we found a significantly higher frequency of R151C, D294H, and T314T variants in family members than in controls (P < 0.05). Preliminary analyses (results not shown) examining the frequency distribution of each MC1R variant according to skin, hair and eye color, skin reactions to sunlight, and nevus phenotypes showed a significantly higher frequency of RHC variants in subjects with red-blonde than dark-brown hair color (P < 0.04), whereas the inverse trend was observed for T314T and R163Q variants (P < 0.03). The D294H variant alone was significantly more frequent in subjects with pale than dark skin color (P = 0.02) and the R163Q variant was less frequent in individuals with high than low or medium propensity to sunburn (P = 0.05). These analyses were also carried out in unaffected subjects, because host-related factors and some MC1R variants have been reported to be risk factors for melanoma by case-control studies. The R151C variant or pooled RHC variants were found to be significantly associated with hair color (P < 0.01), skin color (P < 0.05), and tanning ability (P < 0.03). Combined segregation-linkage analyses of cutaneous malignant melanoma and CDKN2A mutations status were first conducted in the 20 French melanoma-prone families by considering MC1R variants alone (Table 3). Two of the seven

<table>
<thead>
<tr>
<th>Table 1. Genotyped family members by melanoma affection status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2A and MC1R genotypes</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>153 subjects genotyped for both CDKN2A and MC1R CDKN2A, MC1R</td>
</tr>
<tr>
<td>WT, consensus</td>
</tr>
<tr>
<td>WT, one variant</td>
</tr>
<tr>
<td>WT, two variants</td>
</tr>
<tr>
<td>WT, three variants</td>
</tr>
<tr>
<td>Mut, consensus</td>
</tr>
<tr>
<td>Mut, one variant</td>
</tr>
<tr>
<td>Mut, two variants</td>
</tr>
<tr>
<td>Mut, three variants</td>
</tr>
<tr>
<td>13 subjects genotyped for either CDKN2A or MC1R CDKN2A, MC1R</td>
</tr>
<tr>
<td>WT, unknown</td>
</tr>
<tr>
<td>Mut, unknown</td>
</tr>
<tr>
<td>Unk, consensus</td>
</tr>
<tr>
<td>Unk, two variants</td>
</tr>
<tr>
<td>Unk, three variants</td>
</tr>
</tbody>
</table>

Abbreviations: WT, wild type for CDKN2A; Mut, mutation for CDKN2A; unk, unknown genotype for CDKN2A.
*Age at exam is unknown for individuals with unknown melanoma status.

<table>
<thead>
<tr>
<th>Table 2. Frequencies of MC1R variants in members of 20 melanoma-prone pedigrees and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variants</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>V60L</td>
</tr>
<tr>
<td>S63P</td>
</tr>
<tr>
<td>S83L</td>
</tr>
<tr>
<td>D84E</td>
</tr>
<tr>
<td>V92M</td>
</tr>
<tr>
<td>A111V</td>
</tr>
<tr>
<td>R151C</td>
</tr>
<tr>
<td>R151C</td>
</tr>
<tr>
<td>I155T</td>
</tr>
<tr>
<td>R160W</td>
</tr>
<tr>
<td>R163Q</td>
</tr>
<tr>
<td>F196L</td>
</tr>
<tr>
<td>I264I</td>
</tr>
<tr>
<td>D294H</td>
</tr>
<tr>
<td>T314T</td>
</tr>
</tbody>
</table>

*There was no significant difference for each MC1R variant frequency between affected and unaffected members of the 20 melanoma-prone families regardless of CDKN2A genotype.

†Frequencies of R151C, D294H, and T314T were significantly different in 172 controls and in all members of the 20 families (P < 0.044). No difference was found for all other comparisons.
common MC1R variants (frequency $\geq 5\%$ in the family sample) significantly increased CMM risk in carriers of CDKN2A mutations: D294H (OR of hazard function, 3.58; 95% CI, 1.49-8.60; $P = 0.01$) and R163Q (OR, 2.93; 95% CI, 1.16-7.35; $P = 0.04$). Pooled RHC variants increased also significantly CDKN2A penetrance (OR, 2.30; 95% CI, 1.15-4.59; $P = 0.02$). When repeating analyses by considering all genotyped subjects (carriers and noncarriers of CDKN2A mutations), similar results were obtained, except for the R163Q variant, which was only borderline significant ($P = 0.06$).

We then tested for the effect of RHC variants in presence of each host factor significantly associated with melanoma risk (as found in preliminary analyses) in CDKN2A mutation carriers. As seen in Table 4, having at least one RHC variant remained significant in presence of either hair color (OR = 0.05), sunburns ($P = 0.02$), high number of nevi (OR = 0.03), or dysplastic nevi (OR = 0.03). Moreover, each host factor showed an additional significant effect ($P < 0.04$), except for hair color ($P = 0.08$). Similar results were observed in the whole sample of all genotyped subjects (data not shown). We did not consider in these analyses D294H or R163Q, because the number of subjects carrying these variants and having known covariates was too small.

Finally, multivariate analysis (Table 5), examining together RHC variants, host factors, and sun exposure, showed that only RHC variants and dysplastic nevi increased significantly CDKN2A penetrance (OR = 0.03 for RHC variants and OR = 0.01 for dysplastic nevi). The next factor to enter into the model was a high number of nevi, which was borderline significant ($P = 0.053$). Analyses of the sample of carriers and noncarriers of CDKN2A mutations led to similar effects of RHC variants (OR, 1.99; 95% CI, 1.01-3.91; $P = 0.05$) and dysplastic nevi (OR, 3.14; 95% CI, 1.59-6.20; $P < 0.01$) on melanoma risk.

### Discussion

The MC1R gene was reported to be a low-penetrance melanoma predisposing gene as well as a modifier of CDKN2A penetrance especially with respect to RHC variants (22, 23). The present study extends these previous results by showing the significant joint effects of RHC variants and dysplastic nevi on the penetrance of CDKN2A mutations in 20 French melanoma-prone families.

Fourteen MC1R variants were detected in our melanoma-prone families. Seven of these variants (V60L, V92M, R151C, R160W, D294H, R163Q, and T314T) have frequencies higher than 5%. When comparing MC1R allele frequencies observed in Dutch, Australian, and French melanoma-prone families, V60L, V92M, and D294H frequencies were higher in our sample, whereas R151C and R160W frequencies were higher in Australian and Dutch kindreds, respectively (22, 23). The T314T variant has only been detected in French families. Similar results were observed when comparing MC1R allele frequencies between control samples of these three populations. Note that in all three studies, frequency of MC1R variants was higher in the melanoma-prone families than in controls.

Only two (D294H and R163Q) variants of the seven common MC1R variants, considered individually, increased significantly CDKN2A penetrance in our sample. D294H is a RHC variant, being found associated with red-blond hair and fair skin in our sample, and reported to increase melanoma risk by case-controls studies (13, 14, 18). R163Q, weakly associated with fair skin (14, 33) and inversely associated with red-blond hair and sunburn in our sample, was reported by a case-control study to weakly increase melanoma risk (14). Note that the R151C variant, associated with melanoma in case-control studies (13, 14, 18) and in Dutch families segregating CDKN2A mutations (23), did not modify CDKN2A penetrance in our sample.
study. The significant effect of pooled RHC variants in CDKN2A mutation carriers of our sample was similar to that found in the Australian family-based study (22): ORs associated with RHC variants being 2.02 and 2.30 in Australian and French families, respectively.

The increase in melanoma risk according to the number of MC1R variants observed in Dutch p16-Leiden carriers (23) was also found in our sample (results not shown). Indeed, in CDKN2A mutations carriers, the ORs associated with the presence of at least two MC1R variants was 3.58 (95% CI, 1.20-10.67), whereas the OR associated with the presence of only one variant was 1.57 (95% CI, 0.45-5.46). Case-control studies also reported a higher melanoma risk in carriers of two variants rather than just one (13, 14, 18). However, carrying two MC1R variants did not add further risk than carrying one variant in the Australian CDKN2A penetrance analysis (22).

No effect of MC1R genotype on the age at onset for melanoma was observed in the present sample, in agreement with the Dutch study (23) but differing from the Australian study, where a significant decrease in mean age at onset was observed in carriers of both CDKN2A mutation and MC1R variant when compared with carriers of CDKN2A mutation alone (22). These different results may be partly due to a difference in age distribution between samples.

Although case-control studies have indicated that MC1R variants increase melanoma risk independently of pigmentary traits (13, 14, 18), this issue was scarcely investigated in the Australian and Dutch families (22, 23). First, the effect of RHC variants on CDKN2A penetrance remained significant in presence of host-related phenotypes (mainly hair color, sunburns, dysplastic nevi, and high number of nevi). Moreover, each of these latter factors, except hair color, had a significant effect in presence of RHC variants. When examining jointly all potential risk factors, only RHC variants and dysplastic nevi increased significantly CDKN2A penetrance. ORs associated with these two factors were almost unchanged whether the model included either one or both of them, indicating independent effects of RHC variants and dysplastic nevi on CDKN2A penetrance. Analyses done in the sample of carriers and noncarriers of CDKN2A mutations led to similar conclusions. Interestingly, RHC variants had a slightly higher effect on melanoma risk in the subsample of CDKN2A mutation carriers than in the whole sample of genotyped subjects, whereas the inverse trend was observed for dysplastic nevi.

Mechanisms underlying the increased risk of CMM in subjects carrying MC1R variants are not well understood. Functional analyses of different MC1R variants have shown that V60L, R160W, D294H, and R151C variants are unable to stimulate cyclic AMP production as strongly as the wild-type allele, in response to α-MSH stimulation and alter melanin synthesis (34-36). The effect of V92M variant is not well established with discordant results (37, 38). Although no functional analysis was done for R163Q, it was found at high frequency in Asian population (39, 40), suggesting an association with pheomelanin-rich skin. More recently, an evolutionary-based approach, conducted to predict MC1R variants with most likely functional significance, detected R160W and D294H among high-risk variants, whereas V60L, V92M, and R163Q were predicted to be low-risk variants (28).

Because MC1R is known to be involved in the UV sensitivity pathway, MC1R variants may have an effect on human melanocytes response to α-MSH and UV radiation. Indeed, melanocyte cells expressing MC1R variants produce increased levels of pheomelanin leading to limited photoprotection and exhibit increased sensitivity to cytotoxic effects of UV radiation (41).

The modifier effect of MC1R variants on CDKN2A penetrance might suggest a link between p16INK4A and MC1R in response to UV radiation. It has been clearly shown that expression of p16INK4A in human skin cells is increased after UV radiation, suggesting an implication in protective cell cycle response to UV (42); this expression being induced via interactions of α-MSH with MC1R (43). The presence of MC1R variants may alter the protective p16INK4A-mediated cell cycle response to UV and thus increase melanoma risk in carriers of both CDKN2A mutations and MC1R variants.

MC1R variants might also exert a non-UV-related effect on the propensity of melanocytic cellular transformation. It has been recently shown that human melanoma cells overexpressed MC1R compared with normal melanocytes (44), and melanoma cells synthesize and release α-MSH suggesting an autocrine effect of this protein on melanoma cells. It has been shown that α-MSH significantly reduces the proliferation and cellular invasion, through the decrease of fibronectin binding in human melanoma cells expressing the wild-type MC1R, whereas the RHC variants have no effect on growth and adhesion to extracellular matrix specifically with fibronectin (45, 46). Thus, MC1R variants could alter melanogenesis and induce melanoma development and progression via nonpigmentary mechanisms.

In conclusion, this study shows CDKN2A penetrance is modified by MC1R gene variants and presence of dysplastic nevi. It emphasizes the complexity of melanoma pathogenesis, which is likely to involve several pathways (cell cycle pathway, UV sensitivity pathway, nevus pathway, proliferation and invasion pathways, and DNA repair pathway). Investigation of other genes such as those involved in pigmentation, DNA repair, and carcinogen metabolism may help in disentangling these complex underlying mechanisms. Confirmation of these findings in other populations seems warranted. Moreover, such studies may have important consequences to improve the prediction of melanoma risk in families.

Acknowledgments

We thank the participating families, whose cooperation has made this study possible and B. Bachollet, P. Berthet, F. Boitier, J.-P. Cesarini, J. Chevrot-Brenton, O. Dureure, P. Duvillard, F. Grange,
B. Guillot, R. Guimbaud, P. Joly, C. Lasset, J-L. Michel, J-C. Ortoli, B. Sassolas, R. Triller, F. Truchetet, and L. Vermes, as part of the French familial melanoma study group who contributing to the recruitment of families.

References


Melanocortin-1 Receptor (MC1R) Gene Variants and Dysplastic Nevi Modify Penetrance of CDKN2A Mutations in French Melanoma-Prone Pedigrees

Valérie Chaudru, Karine Laud, Marie-Françoise Avril, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/14/10/2384

Cited articles
This article cites 45 articles, 16 of which you can access for free at:
http://cebp.aacrjournals.org/content/14/10/2384.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/14/10/2384.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.