Association of MC1R Variants and Host Phenotypes With Melanoma Risk in CDKN2A Mutation Carriers: A GenoMEL Study

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Background Carrying the cyclin-dependent kinase inhibitor 2A (CDKN2A) germline mutations is associated with a high risk for melanoma. Penetrance of CDKN2A mutations is modified by pigmentation characteristics, nevus phenotypes, and some variants of the melanocortin-1 receptor gene (MC1R), which is known to have a role in the pigmentation process. However, investigation of the associations of both MC1R variants and host phenotypes with melanoma risk has been limited.

Methods We included 815 CDKN2A mutation carriers (473 affected, and 342 unaffected, with melanoma) from 186 families from 15 centers in Europe, North America, and Australia who participated in the Melanoma Genetics Consortium. In this family-based study, we assessed the associations of the four most frequent MC1R variants (V60L, V92M, R151C, and R160W) and the number of variants (1, ≥2 variants) alone or jointly with the host phenotypes (hair color, propensity to sunburn, and number of nevi), with melanoma risk in CDKN2A mutation carriers. These associations were estimated and tested using generalized estimating equations. All statistical tests were two-sided.

Results Carrying any one of the four most frequent MC1R variants (V60L, V92M, R151C, R160W) in CDKN2A mutation carriers was associated with a statistically significantly increased risk for melanoma across all continents (1.24 × 10^4 ≤ P ≤0.0007). A consistent pattern of increase in melanoma risk was also associated with increase in number of MC1R variants. The risk of melanoma associated with at least two MC1R variants was 2.6-fold higher than the risk associated with only one variant (odds ratio = 5.83 [95% confidence interval = 3.60 to 9.46] vs 2.25 [95% confidence interval = 1.44 to 3.52]; \( P_{\text{trend}} = 1.86 \times 10^{-4} \)). The joint analysis of MC1R variants and host phenotypes showed statistically significant associations of melanoma risk, together with MC1R variants (.0001 ≤ P ≤ .04), hair color (.006 ≤ P ≤ .06), and number of nevi (6.9 × 10^4 ≤ P ≤ .02).

Conclusion Results show that MC1R variants, hair color, and number of nevi were jointly associated with melanoma risk in CDKN2A mutation carriers. This joint association may have important consequences for risk assessments in familial settings.

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Cutaneous melanoma, the most common form of melanoma, is a complex disease that arises through multiple etiological pathways. The cyclin-dependent kinase inhibitor 2A (CDKN2A) gene (Mendelian Inheritance in Man 600160), located on chromosome 9p21, is known to be a major high-risk melanoma susceptibility gene that is transmitted according to a dominant mode of inheritance in melanoma-prone families (1,2). It encodes two distinct tumor suppressor proteins that are translated in alternative reading frames (ARFs) from alternate spliced transcripts (3–6). The alpha (α) transcript, comprises exons 1α, 2, and 3 and encodes the p16INK4a protein. This protein is known to inhibit the cyclin-dependent kinase 4 (CDK4)–mediated phosphorylation of retinoblastoma 1 protein and prevents the cell from progressing through the G1 cell cycle checkpoint (3,4). The beta (β) transcript, comprises exons 1β, 2, and 3 and encodes the p14ARF protein. This protein acts via tumor protein p53 pathway to induce cell cycle...
arrest or apoptosis (5,6). The germline CDKN2A mutations have been found in about 40% of melanoma-prone families from around the world (7). Most CDKN2A mutations are scattered through the lengths of exons 1a and 2, thus affecting p16INK4a protein alone or both p16INK4a and p14ARF proteins (7). The penetrance of CDKN2A mutations in multiple-case melanoma families was found to vary across continents, indicating that variations in genetic backgrounds, host characteristics, and/or sun exposure may contribute to the differences in penetrance (8).

Among the host phenotypes that may influence melanoma risk, dysplastic nevi, high numbers of banal nevi, poor tanning ability, and/or propensity to sunburn were shown to be associated with enhanced CDKN2A penetrance in melanoma-prone families (9,10). The melanocortin-1 receptor (MC1R) gene (Mendelian Inheritance in Man 155555), which plays a key role in the pigmentation process (11), has been consistently found to be a low-risk melanoma susceptibility gene in case–control studies, discussed in a review article (12). Moreover, MC1R variants have been shown to increase melanoma risk in families with CDKN2A mutations (13–16).

The MC1R gene is highly polymorphic in populations of European ancestry and more than 85 nonsynonymous variants have been identified (17,18). These include the red hair color (RHC) variants that are consistently associated with red hair, light skin, poor tanning ability, and heavy freckling, and the non-RHC (NRHC) variants that have a weaker, or no association, with red hair (19). In previous studies investigating the associations of MC1R variants with melanoma, whether conducted in case–control series or melanoma-prone families, the melanoma risk was mainly associated with the RHC variants, although it has also been reported to be influenced by certain NRHC variants (12,13–16), indicating that MC1R plays a role in melanoma development beyond that of pigmentation. Moreover, an increase in melanoma risk with an increase in number of MC1R variants has been reported in most (12,14–16), but not all (13,20), studies.

To date, there are limited investigations on the joint associations of MC1R variants, pigmentation, and nevus phenotypes, with melanoma risk in CDKN2A mutation carriers. Two studies (15,16) explored these joint associations (MC1R variants and pigmentation phenotypes, or MC1R variants and nevus phenotypes, or MC1R variants and pigmentation phenotypes and nevus phenotypes) but were conducted in relatively small number of CDKN2A mutation carriers. The joint associations of MC1R variants, pigmentation phenotypes, and/or nevus phenotypes with melanoma risk were tested in one study, but were restricted to RHC variants (16). Moreover, it is not known whether the associations of MC1R variants with melanoma risk in CDKN2A carriers vary, depending on whether the CDKN2A mutation alters p16INK4a protein alone or both p16INK4a and p14ARF proteins. Furthermore, MC1R variants have been inconsistently associated with a reduction in age at diagnosis of melanoma in CDKN2A mutation carriers (13–16).

The Melanoma Genetics Consortium (GenoMEL), which includes major familial melanoma research groups from Europe, North America, and Australia, has recruited the largest sample of CDKN2A mutation carriers, to our knowledge, to assess the association of MC1R variants with melanoma risk. In this study, we analyzed 815 CDKN2A mutation carriers that participated in GenoMEL to assess the association of MC1R variants, alone or in combination with host phenotypes, with melanoma risk in white populations with varying pigmentation characteristics and from countries and continents located at different latitudes with different patterns of sun exposure. We also explored whether the association of MC1R variants with melanoma risk differed if the CDKN2A mutations affected p16INK4a alone or both p16INK4 and p14ARF proteins.

**Subjects and Methods**

**GenoMEL Subjects**

Fifteen GenoMEL centers from Europe, North America, and Australia participated in the present analyses (Appendix). The following geographic locales were defined across three continents: Europe—France (Paris), Italy (Emilia-Romagna and Genoa), the Netherlands (Leiden), Spain (Barcelona), Sweden (Lund, Stockholm), and United Kingdom (Glasgow, Leeds); North America—Boston, NCI, Philadelphia, and Toronto; Australia—Brisbane and Sydney. Individual country locales were only considered within Europe in the analyses.

The CDKN2A mutation carriers from families with at least two cutaneous melanoma patients and presence of a germline CDKN2A

**CONTENTS AND CAVEATS**

**Prior knowledge**

The association between cyclin-dependent kinase inhibitor 2A (CDKN2A) mutations and increased risk of cutaneous melanoma is influenced by host phenotypes (hair color, sunburn, number of nevi), as well as variants of melanocortin-1 receptor (MC1R), a gene associated with pigmentation characteristics and melanoma risk.

**Study design**

The association of MC1R variants, alone or jointly with host phenotypes, with melanoma risk was assessed in a family-based study of CDKN2A mutation carriers using GenoMEL participants from Europe, North America, and Australia.

**Contribution**

Each of the four frequent MC1R variants was associated with an increased melanoma risk in CDKN2A mutation carriers across all continents. The magnitude of the association of MC1R with melanoma increased consistently with an increase in the number of variants. Host phenotypes, analyzed individually or jointly, showed an association with increased melanoma risk. Both hair color and high numbers of nevi were associated with melanoma risk in addition to MC1R variants.

**Implications**

Increased melanoma risk in CDKN2A mutation carriers was associated jointly with host phenotypes and MC1R variants. These results are important for risk assessments in melanoma-prone families.

**Limitations**

The associations presented in this study are based on the sampled families and may not represent the general population.

*From the Editors*
mutation in the family were eligible for the study. The types of CDKN2A mutations identified in the families included in this study are shown in Supplementary Table 1 (available online). Diagnoses of melanoma were confirmed by review of histology and pathology reports, medical records, or death certificates. To be eligible for the study, family members also had to have been genotyped for MC1R; approximately 94.2% of the CDKN2A mutation carriers were genotyped at the MC1R locus and were included in the analyses. For each continent, we checked whether the CDKN2A mutation carriers who were genotyped or not genotyped for MC1R differed in terms of melanoma affection status, sex, and age at examination and found that there was no difference (data not shown). Table 1 presents the total number of participants from 186 families (N = 815; 473 melanoma patients [affected]), and 342 unaffected relatives with no manifestation of melanoma at the time the study was initiated) who were genotyped at the CDKN2A and MC1R loci by the GenoMEL study centers. The degree of familial relationship was estimated by examining all possible pairs of affected and unaffected subjects within families and their distribution was as follows—22% of affected and unaffected pairs were first-degree relatives, 23% were second-degree relatives, 20% were third-degree relatives, and the remaining 35% were more remote relatives. For all centers, written informed consent was obtained from all subjects before recruitment under an Institutional Review Board–approved protocol. Although the process of identifying and recruiting families differed among the GenoMEL centers because of variation in local health-care procedures and/or approaches for accruing families, the eligibility criteria for inclusion in this study were uniform, as described above. Details of the participating families and data collection have been described elsewhere (see Table 1 for references).

Genotyping of CDKN2A and MC1R

The protocol for detecting CDKN2A mutations has been described elsewhere; most families participating in the GenoMEL consortium were recently evaluated for the types of CDKN2A mutations and associated clinical factors, including age at melanoma diagnosis and presence of multiple primary melanomas in the family (7,30,31). The distribution of the CDKN2A mutations identified in the 186 families included in this study and their effect on p16INK4a and p14ARF proteins are shown in Supplementary Table 1 (available online).

The MC1R genotyping was performed in each center by sequencing the entire open reading frame of the single-exon gene in both affected patients and unaffected subjects. The methods varied slightly between the centers, and examples of methods can be found in Vajdic et al. (32) and Kanetsky et al. (33). We checked that the distribution of MC1R variants were in Hardy–Weinberg equilibrium; no departure from Hardy–Weinberg equilibrium at the 5% significance level was found. The Mendelian inconsistencies of the MC1R genotypes were checked using the PedCheck program (34), and any inconsistent genotypes were coded as missing data.

Table 1. Cyclin-dependent kinase inhibitor 2A (CDKN2A) mutation carriers from the Melanoma Genetics Consortium (GenoMEL) and melanoma affection status in the CDKN2A mutation carriers genotyped for melanocortin-1 receptor (MC1R)* gene

| Consortium center       | No. of families; geographic locale, continent | CDKN2A mutation carriers genotyped for MC1R | No. of melanoma patients‡ | No. of unaffected subjects‡ |
|-------------------------|-----------------------------------------------|---------------------------------------------|----------------------------|-----------------------------|
| Europe                  |                                               | No. of CDKN2A mutation carriers | No. of melanoma patients | No. of unaffected subjects |
| Paris                   | France, Europe                                | 27 (16)                                    | 95                         | 52                          | 43                          |
| Emilia-Romagna          | Italy, Europe                                 | 4 (21)                                     | 8                          | 6                           | 2                           |
| Genoa                   | Italy, Europe                                 | 14 (22)                                    | 38                         | 26                          | 12                          |
| Leiden                  | the Netherlands, Europe                       | 8 (14)                                     | 113                        | 52                          | 61                          |
| Barcelona               | Spain, Europe                                 | 16 (23)                                    | 66                         | 30                          | 36                          |
| Lund                    | Sweden, Europe                                | 9                                          | 33                         | 17                          | 16                          |
| Stockholm               | Sweden, Europe                                | 4 (24)                                     | 15                         | 5                           | 10                          |
| Glasgow                 | United Kingdom, Europe                        | 13 (25)                                    | 27                         | 21                          | 6                           |
| Leeds                   | United Kingdom, Europe                        | 34 (26)                                    | 102                        | 61                          | 41                          |
| Subtotal                |                                              | 129                                        | 497                        | 270                         | 227                         |
| North America           |                                               |                                            |                            |                             |                             |
| Boston                  | USA, North America                            | 6 (27)                                     | 15                         | 11                          | 4                           |
| NCI                     | USA, North America                            | 16 (15)                                    | 136                        | 70                          | 66                          |
| Philadelphia            | USA, North America                            | 2                                          | 10                         | 9                           | 1                           |
| Toronto                 | Canada, North America                         | 15                                         | 32                         | 26                          | 6                           |
| Subtotal                |                                              | 39                                         | 193                        | 116                         | 77                          |
| Australia               |                                               |                                            |                            |                             |                             |
| Brisbane                | Australia                                     | 17 (28)                                    | 96                         | 76                          | 20                          |
| Sydney                  | Australia                                     | 1 (29)                                     | 29                         | 11                          | 18                          |
| Subtotal                |                                              | 18                                         | 125                        | 87                          | 38                          |
| Total                   |                                              | 186                                        | 815                        | 473                         | 342                         |

* The protocol for detecting CDKN2A mutations has been described elsewhere (7,30,31). MC1R genotyping was performed by sequencing the entire open reading frame of the single-exon gene (32,33). NCI = National Cancer Institute.
† The median ages of melanoma patients at examination were 47 years in Europe, 42 years in North America, and 54 years in Australia; and the median ages at diagnosis were 36 years in Europe, 31 years in North America, and 34 years in Australia.
‡ The median ages of unaffected subjects at examination were 41 years in Europe, 34 years in North America, and 47 years in Australia.
Data Collection

Information on familial relationships among members of the same family; demographic characteristics (sex; date of birth; and age at death and cause of death, if deceased); melanoma status (affected vs unaffected) together with confirmation of melanoma diagnosis and age at diagnosis; CDKN2A mutation status (no mutation [homozygote wild type] vs presence of a mutation [heterozygote or homozygote for the mutation]) and, in mutation carriers, location of the mutation in CDKN2A locus (promoter region, exons 1α, 1β, 2 and 3, and introns); CDKN2A nucleotide change and subsequent change in amino acid in p16INK4a and p14ARF proteins; MC1R genotypes (wild-type homozygotes for the consensus sequence, heterozygotes or homozygotes for a variant at each position of the sequence where an MC1R variant had been detected across all participating GenoMEL centers), pigmentation characteristics, and nevus phenotypes (including hair color, propensity to sunburn, nevus count) were obtained from each center using a standardized format based on a uniform coding scheme across GenoMEL centers. The markers of pigmentation and nevus phenotypes used in this study were coded as follows—hair color (classified as red, blond, brown, black); skin reaction to sun exposure (never burns, sometimes burns, usually burns, always burns); and nevus count (none, few, some and many nevi). The data received from each center were integrated into a common dataset using the Statistical Analysis System (SAS) software (version 9.1, developed by SAS Institute Inc, Cary, NC).

Statistical Analysis

Associations between MC1R variants and melanoma affection status (affected vs unaffected) were evaluated in CDKN2A mutation carriers using the generalized estimating equations (GEE) method to take into account familial dependences. The GEE method, a semiparametric regression method, specifies the relationship between the disease outcome (melanoma affection status) and predictor variables (eg, MC1R variants, host phenotypes) through a link function and takes into account the correlations among disease outcomes of family members through a correlation matrix. We used the logit link function and exchangeable correlation matrix, which assumes equal correlations among the disease outcomes in family members, to estimate odds ratios (ORs) and 95% confidence intervals (CIs). All analyses were adjusted for sex and age at examination as a continuous variable. We used age at examination for all subjects rather than age at diagnosis for melanoma patients, unaffected subjects, and all subjects) and age at examination as a continuous variable. We used age at diagnosis; age at examination; and the locale indicator variable. These interaction terms, which are equal to zero under the null hypothesis of homogeneity of the association of melanoma with any of the MC1R variables analyzed among geographic locales (as defined in the GenoMEL subjects paragraph) was tested by introducing MC1R variable x locale interaction terms in the regression model between disease outcome and predictor variables. This locale adjustment in the regression model can correct for potential population stratification because the locale variable was country specific for European mutation carriers in all analyses, whereas it was continent specific for North American and Australian mutation carriers because they were all of European ancestry and more than 70% were recruited from a single center, respectively (15, 28). The homogeneity in the association of melanoma with any of the MC1R variables analyzed among geographic locales (as defined in the GenoMEL subjects paragraph) was tested by introducing MC1R variable x locale interaction terms in the regression model between melanoma and the MC1R variable, which also included sex, age at examination, and the locale indicator variable. These interaction terms, which are equal to zero under the null hypothesis of homogeneity of the association of MC1R variable with melanoma across geographic locales, were tested using a generalized score test which follows a χ² distribution with number of degrees of freedom equal to number of locales minus 1 for a given MC1R variable category. It must be noted that, for MC1R variables with only one category (eg, presence of a given MC1R variant or presence of any MC1R variant), there was only one interaction term per geographic locale, whereas for MC1R variables with more than one category (eg, number of MC1R variants and types of variants), there was one interaction term between each MC1R variable category and each geographic locale. We also used the Cochran Q test (35) to test for homogeneity of the estimates of the odd -ratios associated with each of the MC1R variables among European, North American, and Australian mutation carriers. We also investigated whether the associations of the frequent MC1R variants and number of MC1R variants with melanoma risk were the only ones that were analyzed individually.

We first investigated associations between melanoma and each frequent nonsynonymous MC1R variant individually by comparing carriage of at least one variant (homozygotes and heterozygotes pooled) to homozygosity for the MC1R consensus sequence (reference category). Because many MC1R variants were too rare to examine their individual association with melanoma risk in CDKN2A carriers, all nonsynonymous variants were grouped in various ways to make the following comparisons—carriers of any MC1R variant compared with homozygosity for the MC1R consensus sequence; carriers of multiple MC1R variants (1, ≥2 variants) compared with homozygosity for the MC1R consensus sequence; and carriers of specific types of MC1R variants (1 NRHC variant, 1 RHC variant, ≥2 NRHC variants, ≥2 RHC variants, or carriers of both RHC and NRHC variants) compared with MC1R consensus sequence. The RHC variants included four MC1R variants (R151C, R160W, D294H, and D84E), which have been consistently reported to be associated with RHC and light skin color; all other nonsynonymous variants were coded as NRHC.

Generalized score tests for association of melanoma risk with carriage of any MC1R variant and number of variants were conducted for each geographic locale separately, whereas tests for other MC1R variables (individual variants and types of variants) were conducted by continent (ie, pooling locales within Europe) because of small sample size in individual European countries. Pooled analyses of all locales within Europe and across continents were carried out by introducing a locale indicator variable in the regression model between disease outcome and predictor variables. This locale adjustment in the regression model can correct for potential population stratification because the locale variable was country specific for European mutation carriers in all analyses, whereas it was continent specific for North American and Australian mutation carriers because they were all of European ancestry and more than 70% were recruited from a single center, respectively (15,28). The homogeneity in the association of melanoma with any of the MC1R variables analyzed among geographic locales (as defined in the GenoMEL subjects paragraph) was tested by introducing MC1R variable x locale interaction terms in the regression model between melanoma and the MC1R variable, which also included sex, age at examination, and the locale indicator variable. These interaction terms, which are equal to zero under the null hypothesis of homogeneity of the association of MC1R variable with melanoma across geographic locales, were tested using a generalized score test which follows a χ² distribution with number of degrees of freedom equal to number of locales minus 1 for a given MC1R variable category. It must be noted that, for MC1R variables with only one category (eg, presence of a given MC1R variant or presence of any MC1R variant), there was only one interaction term per geographic locale, whereas for MC1R variables with more than one category (eg, number of MC1R variants and types of variants), there was one interaction term between each MC1R variable category and each geographic locale. We also used the Cochran Q test (35) to test for homogeneity of the estimates of the odd -ratios associated with each of the MC1R variables among European, North American, and Australian mutation carriers. We also investigated whether the associations of the frequent MC1R variants and number of MC1R variants with melanoma risk...
Table 2. Frequency of melanocortin-1 receptor (MC1R) variants in cyclin-dependent kinase inhibitor 2A (CDKN2A) mutation carriers analyzed by continent and in all three continents*

| Consensus sequence‡ | All continents | Consensus sequence‡ | All continents |
|---------------------|----------------|---------------------|----------------|
| Consensus sequence‡ | None | Consensus sequence‡ | None |
| Nonsynonymous frequent variants||Nonsynonymous frequent variants||
| g.178G>T | V60L | 123 | 12.4 | 63 | 16.3 | 26 | 10.4 | 212 | 13.0 |
| g.274G>A | V92M | 105 | 10.5 | 29 | 7.5 | 21 | 8.4 | 155 | 9.5 |
| g.451C>T | R151C | 116 | 11.7 | 40 | 10.4 | 61 | 24.4 | 217 | 13.3 |
| g.478C>T | R160W | 94 | 9.5 | 33 | 8.5 | 17 | 6.8 | 144 | 8.8 |

| Nonsynonymous rare variants¶| Nonsynonymous rare variants¶|
| g.44A>G | N15S | 0 | 0 | 0 | 0 | 1 | 0.4 | 1 | 0.1 |
| g.206A>C | H69P | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.247T>C | S83P | 0 | 0 | 4 | 1.0 | 0 | 0 | 4 | 0.3 |
| g.248C>T | S83L | 2 | 0.2 | 0 | 0 | 0 | 0 | 2 | 0.1 |
| g.252C>A | D84E | 9 | 0.9 | 6 | 1.6 | 2 | 0.8 | 17 | 1.0 |
| g.284C>T | T95M | 3 | 0.3 | 0 | 0 | 0 | 0 | 3 | 0.2 |
| g.364G>A | V122M | 2 | 0.2 | 0 | 0 | 0 | 0 | 2 | 0.1 |
| g.383T>A | M128K | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.425G>A | R142H | 5 | 0.5 | 1 | 0.3 | 0 | 0 | 6 | 0.4 |
| g.456C>A | Y152X | 2 | 0.2 | 0 | 0 | 1 | 0.4 | 17 | 1.0 |
| g.512C>G | A171G | 51 | 5.1 | 12 | 3.1 | 11 | 4.4 | 74 | 4.5 |
| g.596T>C | F196L | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.652G>A | A218T | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.653C>G | A218G | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.669C>G | A240A | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.720T>C | I221T | 2 | 0.2 | 0 | 0 | 0 | 0 | 2 | 0.1 |
| g.835A>G | N279D | 5 | 0.5 | 0 | 0 | 0 | 0 | 5 | 0.3 |
| g.880G>C | D294H | 21 | 2.1 | 16 | 4.2 | 8 | 3.2 | 45 | 2.8 |

| Insertions| Insertions|
| g.86_87insA | 1 | 0.1 | 5 | 1.3 | 0 | 0 | 6 | 0.4 |
| g.537_538insC | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |

| Synonymous frequent variants||Synonymous frequent variants||
| g.942A>G | T314T | 102 | 10.3 | 41 | 10.7 | 9 | 3.7 | 152 | 9.3 |

| Synonymous rare variants¶| Synonymous rare variants¶|
| g.102G>C | R34R | 0 | 0 | 1 | 0.3 | 0 | 0 | 1 | 0.1 |
| g.399C>T | C133C | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.637C>A | R213R | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| g.699G>A | Q233Q | 2 | 0.2 | 0 | 0 | 0 | 0 | 2 | 0.1 |
| g.720T>C | A240A | 0 | 0 | 1 | 0.3 | 0 | 0 | 1 | 0.1 |

(Table continues)
Table 2 (continued).

| MC1R nucleotide change | MC1R amino acid change | GenoMEL Participants Included in the Study and MC1R Variants |
|------------------------|------------------------|-------------------------------------------------------------|
|                        |                       | Results                                                      |
|                        |                       | No. of chromosomes† (n = 994)  | No. of chromosomes† (n = 386)  | No. of chromosomes† (n = 250)  | No. of chromosomes† (n = 1630) |
| g.792C>T               | I264I                 | % of chromosomes with and without MC1R variant†             | % of chromosomes with and without MC1R variant† | % of chromosomes with and without MC1R variant† | % of chromosomes with and without MC1R variant† |
| g.949C>T               | S316S                 | 0                                                          | 0.8                                       | 0                                                          | 0.4                                      |

* The frequency of MC1R variants was estimated in all CDKN2A mutation carriers genotyped for MC1R. (N = 815: 497 from Europe, 193 from North America, and 125 from Australia).
† The number of chromosomes was twice the number of CDKN2A mutation carriers genotyped for MC1R.
‡ The number of chromosomes carrying no MC1R variant (consensus sequence) and those carrying a given variant are shown for all CDKN2A mutation carriers per continent and in all three continents.
§ The proportions shown in this table are the number of chromosomes carrying no MC1R variant (consensus sequence) or a given variant divided by the total number of chromosomes in CDKN2A mutation carriers per continent and in all three continents.
¶ The rare variants shown in this table are those with an estimated frequency greater than or equal to 5% in CDKN2A mutation carriers from at least one continent and all three continents.
¶ The rare variants shown in this table are those with an estimated frequency less than 5% in CDKN2A mutation carriers from all three continents.
statistically significant difference in the median age at diagnosis of melanoma: 36 years in Europe, 31 years in North America, and 34 years in Australia (P = .08) was observed.

We estimated the frequency of each MC1R variant in CDKN2A mutation carriers from each continent and from all three continents (Table 2; details provided in Supplementary Table 2, available online). A total of 33 variants of MC1R were detected; 23 variants corresponded to nonsynonymous amino acid changes, eight variants corresponded to synonymous amino acid changes, and two variants corresponded to insertions. All subsequent analyses were restricted to nonsynonymous variants. Four nonsynonymous variants (V60L, V92M, R151C, and R160W) were observed at a frequency greater than or equal to 5% in at least one continent and all three continents in all mutation carriers (Table 2) as well as in affected and unaffected carriers (Supplementary Table 2, available online). The frequency of these variants did not differ statistically significantly across continents in unaffected mutation carriers (P > .20).

**Associations of MC1R Variants With Melanoma Risk in CDKN2A Mutation Carriers**

We first assessed the association of each of the four most frequent MC1R variants with melanoma risk in CDKN2A carriers. Both RHC (R151C and R160W) and NRHC (V60L and V92M) variants were associated with increased melanoma risk, but with varying strengths in different continents (Table 3). In Europe, this association was statistically significant for each variant, and the strongest association was noted for RHC variants, followed by NRHC variants (.0002 ≤ P ≤ .03). In North America, the association with increased melanoma risk reached statistical significance with R151C, R160W, and V92M variants (.05 ≤ P ≤ .02), whereas in Australia, the association reached statistical significance with V60L (P = .009) and R151C (P = .03) variants. The association of any of these MC1R variants with melanoma risk did not show evidence of heterogeneity across continents (P_homogeneity ≥ .09). The pooled estimates of the odds ratios adjusted for age, sex, and locale were always higher than 2.0 and were lowest for V92M (OR = 2.43, 95% CI = 1.45 to 4.06) and highest for R151C (OR = 4.68, 95% CI = 2.52 to 8.68).

We carried out subsequent analyses by pooling all nonsynonymous variants in different ways—pooling all of them in one category (presence of any variant), pooling them according to their number (1, ≥2 variants), and pooling them according to their number and type (number of RHC and NRHC variants). When all nonsynonymous MC1R variants were pooled (Table 3), carrying at least one variant was associated with increased melanoma risk in all three continents (.0006 ≤ P ≤ .05). Some variation in the increase in melanoma risk was observed within Europe (Supplementary Table 3, available online). The highest increases in risk were observed in France, Spain, and Sweden (.02 ≤ P ≤ .04), whereas the odds ratio was close to unity in Italy, the country with the smallest sample size (Supplementary Table 3, available online). Nevertheless, tests for heterogeneity in the association of at least one MC1R variant with melanoma risk among geographic locales were not statistically significant (P = .11 within Europe; and P = .16 across all continents). In CDKN2A mutation carriers from all three continents, the presence of at least one MC1R variant was associated with a threefold increase in melanoma risk (OR = 3.05, 95% CI = 1.99 to 4.67). We then investigated whether there was an increase in melanoma risk associated with an increase in the number of MC1R variants. The increase in the number of MC1R variants showed a consistent increase in melanoma risk in CDKN2A mutation carriers from Europe, North America, and Australia (for Europe, P_mixed = 6.25 × 10⁻⁴; for North America, P_mixed = .01; and for Australia, P_mixed = .03) (Table 3). This increase in risk with the increase in numbers of MC1R variants was seen in all European countries except Italy, where there was no increase in melanoma risk associated with either one MC1R variant or at least two variants (Supplementary Table 3, available online), and in Sweden, where the increase in melanoma risk associated with one variant was similar to the increase in risk associated with at least two variants (Supplementary Table 3, available online). No statistically significant heterogeneity for the increase in melanoma risk with the increase in number of MC1R variants was detected either across European countries (P_homogeneity = .14) or across all continents (P_homogeneity = .23). Overall, the risk associated with at least two MC1R variants was 2.6-fold higher than the risk associated with only one variant (OR = 5.83 [95% CI = 3.60 to 9.46] vs 2.25 [95% CI = 1.44 to 3.52]; P_mixed = 1.86 × 10⁻⁴). Next, we explored whether there was an increase in melanoma risk with both the number and types of MC1R variants (RHC variants and NRHC variants). We observed that in all continents an increase in melanoma risk was associated with an increase in number of variants and, mostly, when the genotype included RHC variants (Table 3). The association with number and types of MC1R variants was homogeneous among continents (P_homogeneity = .07). The odds ratios estimated from all continents showed that there was a more than fivefold increase in melanoma risk in CDKN2A mutation carriers with at least two RHC variants compared with one NRHC variant (OR = 11.78 [95% CI = 5.34 to 26.02] vs 2.08 [95% CI = 1.28 to 3.40], respectively).

Because there was no statistically significant evidence for heterogeneity in the association of any of the studied MC1R variables with the melanoma risk across all geographic locales, we investigated whether the association of melanoma risk with individual MC1R variants and number of variants differed by the type of CDKN2A mutation in all CDKN2A mutation carriers from all three continents, while adjusting for age, sex, and geographic locale (Table 4). Overall, the point estimates of the odds ratios associated with each MC1R variant, except R160W, were higher when the CDKN2A mutations affected the p16INK4a protein alone, than when they affected both p16INK4a and p14ARF proteins. However, the confidence intervals were wide and tests of homogeneity for any MC1R variant according to the type of CDKN2A mutation were not significant (P ≥ .09). Similar results were obtained when the analysis was done with the number of MC1R variants (Table 4).

**Joint Associations of MC1R Variants and Host Phenotypes With Melanoma Risk in CDKN2A Mutation Carriers**

Next we assessed the joint associations of MC1R variants and host phenotypes with melanoma risk in all CDKN2A mutation carriers from all three continents. We confirmed that, in the unaffected subjects, R151C and R160W variants were statistically significantly
Table 3. Association of individual melanocortin-1 receptor (MC1R) variants, number of MC1R variants, and types of MC1R variants with melanoma risk in cyclin-dependent kinase inhibitor 2A (CDKN2A) mutation carriers analyzed by continent and in all three continents*

| MC1R variants | Europe | North America | Australia | All continents |
|---------------|--------|---------------|-----------|---------------|
|               | No. of participants affected/ unaffected† | OR (95% CI)‡ | P | No. of participants affected/ unaffected† | OR (95% CI)‡ | P | No. of participants affected/ unaffected† | OR (95% CI)‡ | P | No. of participants affected/ unaffected† | OR (95% CI)‡ | P | P_homogeneity ||
| Individual MC1R variants | | | | | | | | | | | | | |
| V60L | 109/120 | 2.50 | (1.40 to 4.66) | .003 | 50/41 | 4.45 | (1.36 to 14.56) | .09 | 32/13 | 6.91 | (2.10 to 22.75) | .009 | 191/174 | 3.42 | (2.10 to 5.58) | .38 |
| V92M | 91/117 | 2.03 | (1.10 to 3.74) | .03 | 28/35 | 4.03 | (1.08 to 14.98) | .03 | 24/17 | 2.51 | (1.85 to 7.41) | .12 | 143/169 | 2.43 | (1.45 to 4.06) | .25 |
| R151C | 115/104 | 4.31 | (1.81 to 10.23) | .0002 | 36/37 | 6.23 | (2.25 to 17.28) | .02 | 57/20 | 5.58 | (1.56 to 20.0) | .03 | 208/161 | 4.68 | (2.52 to 8.68) | .73 |
| R160W | 94/105 | 3.32 | (1.77 to 6.21) | .001 | 35/32 | 15.04 | (3.20 to 70.67) | .05 | 23/14 | 2.52 | (0.97 to 6.57) | .33 | 152/151 | 4.13 | (2.30 to 7.43) | .09 |
| Any MC1R variant | 270/227 | 2.59 | (1.57 to 4.28) | .0006 | 116/77 | 5.67 | (2.1 to 15.29) | .05 | 87/38 | 4.04 | (1.53 to 10.65) | .02 | 472/342 | 3.05 | (1.99 to 4.67) | .16 |
| No. of MC1R variants | 270/227 | 116/77 | 270/227 | 472/342 | | | | | |
| 1 | 1.85 | (1.09 to 3.15) | .02 | 3.93 | (1.36 to 11.32) | .06 | 3.73 | (1.67 to 8.30) | .02 | 2.25 | (1.44 to 3.52) | .009 |
| ≥2 | 4.46 | (2.58 to 7.57) | 1.32 × 10^{-5} | 13.57 | (4.94 to 37.29) | .02 | 5.09 | (1.25 to 20.74) | .03 | 5.83 | (3.60 to 9.46) | 9.66 × 10^{-8} |
| P_homogeneity | | | | | 6.25 × 10^{-6} | | | | | | | | | | | |
| Types of MC1R variants | 270/227 | 116/77 | 270/227 | 472/342 | | | | | |
| 1 NRHC# | 1.75 | (1.99 to 3.10) | .05 | 3.54 | (1.22 to 10.25) | .04 | 2.55 | (1.66 to 9.84) | .16 | 2.08 | (1.28 to 3.40) | .003 |
| 1 RHC** | 2.04 | (1.08 to 3.85) | .03 | 4.36 | (1.21 to 15.75) | .09 | 4.74 | (1.41 to 15.99) | .02 | 2.59 | (1.47 to 4.57) | .002 |
| ≥2 NRHC | 2.74 | (1.31 to 5.77) | .01 | 8.00 | (2.33 to 27.41) | .03 | 1.86 | (1.55 to 6.28) | .33 | 3.62 | (1.90 to 6.89) | .0002 |
| 1 RHC, 1 NRHC | 4.43 | (2.31 to 8.49) | 4.9 × 10^{-5} | 11.13 | (3.44 to 35.97) | .01 | 6.12 | (1.03 to 36.28) | .04 | 6.24 | (3.43 to 11.34) | 9.97 × 10^{-8} |
| ≥2 RHC | 7.73 | (3.61 to 16.58) | 7.6 × 10^{-5} | 7.72 | (1.30 to 45.75) | .03 | 11.78 | (5.34 to 26.02) | .07 |

* The association of each MC1R variable (individual MC1R variants, any MC1R variant, number of MC1R variants, types of MC1R variants) with melanoma risk was estimated by using homozygosity for the MC1R consensus sequence as the reference category. CI = confidence interval; OR = odds ratio; NRHC = nonred hair color; RHC = red hair color.

† The number of GenoMEL participants contributing to the analysis of a given MC1R variable (individual MC1R variants, any MC1R variant, number of MC1R variants, types of MC1R variants) that were affected with melanoma and their unaffected relatives.

‡ The odds ratios and 95% confidence intervals are measures of association between melanoma risk and MC1R variants. These odds ratios were estimated by the generalized estimating equations method using a logit link function and an exchangeable correlation matrix to take into account the correlations among the family members' melanoma affection status (affected, unaffected). The odds ratios shown in this table are adjusted for age, sex, and geographic locales.

§ P values for the two-sided generalized score test of association between melanoma risk and MC1R variants.

¶ P values for the two-sided generalized score test of homogeneity of the association of MC1R variants with melanoma risk among different geographic locales.

# Nonsynonymous MC1R variants that were not RHC variants.

** RHC variants included R151C, R160W, D294H, and D84E.

| Participants | No. of participants, affected/ unaffected† | OR (95% CI)‡ | P |
|--------------|------------------------------------------|---------------|---|
| Europe | 116/77 | 5.67 | .03 |
| North America | 87/38 | 4.04 | .05 |
| Australia | 472/342 | 3.05 | .07 |
| All continents | 473/342 | 1.99 | .67 |

P values for the two-sided trend test which tests for a change in melanoma risk with a linear increase in the number of MC1R variants (0, 1, ≥2).
associated with red or blond hair color (P = .016) and the R151C (P = .002), V60L (P = .03), and V92M (P = .03) variants were statistically significantly associated with sunburn, but no variant was associated with high numbers of nevi (data not shown). Table 5 shows that when each host phenotype was analyzed separately, hair color, sunburn, and high numbers of nevi were statistically significantly associated with increase in melanoma risk, while adjusting for age, sex, and geographic locale (for red or blond hair color: OR = 3.30, 95% CI = 1.98 to 5.52; for usually or always burns: OR = 2.10, 95% CI = 1.39 to 3.17; and for high numbers of nevi: OR = 3.05, 95% CI = 1.95 to 4.78). In the presence of host phenotypes, analyzed individually or jointly, the increase in melanoma risk with any one of the four frequent MC1R variants remained statistically significant (Table 5). Hair color showed an additional association with melanoma risk with most variants (.01 ≤ P ≤ .07), whereas sunburn was only marginally statistically significant (P ≥ .03) (Table 5). Moreover, an increase in the number of nevi contributed independently to melanoma risk (.005 ≤ P ≤ .02). We obtained similar results when the number of variants was analyzed. A statistically significant increase in melanoma risk with number of variants in the presence of each host phenotype or with all phenotypes was observed (for one variant, .01 ≤ P ≤ .02; and for at least two variants, 1.6 × 10⁻¹ ≤ P ≤ .0001). Hair color and number of nevi, both separately and together, showed statistically significant associations with melanoma risk in addition to the number of MC1R variants (for hair color, P ≤ .006; and for nevi, P ≤ 2.6 × 10⁻¹), whereas the association with sunburn was no longer statistically significant (P ≥ .11). Because MC1R variants are much more strongly associated with red hair than with blond hair in the general population (36,37), analyses were repeated after excluding the 57 CDKN2A mutation carriers with red hair and showed associations between MC1R variants and hair color with melanoma risk similar to those shown in Table 5, indicating that blond hair was the major determinant of the odds ratios that were previously obtained when subjects with red hair were included in the analysis (data not shown). Further stratified analysis based on hair color showed that the statistically significant increase in melanoma risk that was associated with individual MC1R variants or number of variants was limited to subjects with brown or black hair (Supplementary Table 4, available online), thus confirming the role of MC1R beyond that due to pigmentation.

Association of MC1R Variants With Age at Diagnosis of Melanoma

Finally, to assess whether MC1R variants have an influence on age at diagnosis of melanoma, we examined the median ages at diagnosis of melanoma according to various categories of MC1R variables (individual variants, presence of any variant, number of variants, and number and types of variants). As shown in Table 6, there was a slight decrease in age at diagnosis in CDKN2A mutation carriers with presence of R151C or R160W variants or with increase in number and types of variants. This decrease in age at diagnosis reached marginally statistical significance in CDKN2A mutation carriers from Europe (.02 ≤ P ≤ .05), or when the CDKN2A mutation carriers were pooled across all continents (.008 ≤ P ≤ .06).

Discussion

This study investigated the associations of MC1R variants with melanoma risk in 815 CDKN2A mutation carriers from 186 families that participated in 15 GenoMEL centers across Europe, North America, and Australia. The included families had at least two cutaneous melanoma patients and the presence of a germline CDKN2A mutation in the family. To our knowledge, it represents...
Table 5. Association of host phenotypes and melanocortin-1 receptor (MC1R) variants with melanoma risk in cyclin-dependent kinase inhibitor 2A (CDKN2A) mutation carriers from all continents*

| Host phenotypes alone | Joint associations of host phenotypes and individual MC1R variants | Joint associations of host phenotypes and number of MC1R variants |
|-----------------------|---------------------------------------------------------------|-------------------------------------------------|
|                       | Host phenotypes and individual MC1R variants                  | Host phenotypes and No. of MC1R variants           |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
|                       | PS                                           | PS                                           | PS                                           |
| Hair color (1.98 to 5.52) | Hair color (1.10 to 7.50) | Hair color (1.19 to 9.77) | Hair color (1.34 to 6.87) |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.16 to 5.62) | 2.89 (1.60 to 4.16) | 3.41 (2.68 to 4.37) | 3.07 (2.14 to 4.37) |
| Sunburn (1.39 to 3.17)    | Sunburn (1.79 to 3.26)  | Sunburn (1.05 to 5.89) | Sunburn (1.15 to 5.41) |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.15 to 5.62) | 2.95 (1.60 to 4.94) | 4.06 (2.25 to 6.66) | 2.67 (1.21 to 4.17) |
| Nevi (1.95 to 4.78)     | Nevi (1.49 to 6.91)    | Nevi (1.34 to 5.93)  | Nevi (1.11 to 4.17)   |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.14 to 5.65) | 2.88 (1.55 to 5.50) | 4.88 (2.25 to 6.66) | 2.48 (1.21 to 4.17) |
| Hair color (1.44 to 3.79) | Hair color (1.13 to 8.19) | 3.05 (1.28 to 10.56) | Hair color (1.25 to 7.53) |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.15 to 6.82) | 2.48 (1.55 to 6.82) | 3.03 (1.40 to 5.49) | 2.66 (1.23 to 5.78) |
| Sunburn (1.39 to 3.08)  | Sunburn (1.66 to 2.81)   | Sunburn (1.92 to 5.67) | Sunburn (1.12 to 5.43) |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.12 to 4.88) | 2.48 (1.55 to 6.82) | 3.03 (1.40 to 5.49) | 2.66 (1.23 to 5.78) |
| Nevi (1.91 to 4.31)     | Nevi (1.55 to 6.82)    | Nevi (1.61 to 6.56)  | Nevi (1.18 to 4.81)   |
|                       | OR (95% CI)‡                                      | OR (95% CI)‡                                      | OR (95% CI)‡                                      |
| ≥1 individual MC1R variant (1.19 to 4.31) | 2.48 (1.55 to 6.82) | 3.03 (1.40 to 5.49) | 2.66 (1.23 to 5.78) |

* The associations of host phenotypes with melanoma risk were estimated by using the following dichotomous categories: hair color (blond or red vs dark or brown), propensity to sunburn (usually or always burns vs sometimes or never burns) and nevus count (some or many nevi vs none or few nevi). The association of each MC1R variable (individual MC1R variants, number of MC1R variants) with melanoma risk was estimated by using homozygosity for the MC1R consensus sequence as the reference category. CI = confidence interval; OR = odds ratio.

† The number of affected (melanoma patients) and unaffected relatives contributing to a given analysis (host phenotypes alone, host phenotypes and individual MC1R variants, and host phenotypes and number of MC1R variants) are shown in parentheses; only the subjects who have all their host phenotypes known have been included in these analyses.

‡ The odds ratios and 95% confidence intervals were estimated by the generalized estimating equations method. The odds ratios were adjusted for age, sex, and geographic locales.

§ P values for the two-sided generalized score test of association between melanoma risk and any predictor variable (host phenotypes and/or MC1R variants).
Table 6. Association of melanocortin-1 receptor (*MC1R*) variants with median ages at diagnosis of melanoma in case patients carrying cyclin-dependent kinase inhibitor 2A (*CDKN2A*) mutations*

| MC1R variant | Europe | North America | Australia | All continents |
|--------------|--------|---------------|-----------|---------------|
|              | No. of melanoma patients | Median age at diagnosis | No. of melanoma patients | Median age at diagnosis | No. of melanoma patients | Median age at diagnosis | No. of melanoma patients | Median age at diagnosis |
| Individual MC1R variants |        |               |           |               |               |                        |                       |                           |
| V60L         | 108    | 39            | 50        | 32            | 190           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| V92M         | 91     | 39            | 28        | 24            | 143           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| R151C        | 115    | 39            | 35        | 57            | 207           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| R160W        | 94     | 39            | 35        | 23            | 152           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| Any MC1R variant | 268    | 39            | 115       | 87            | 470           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| No. of MC1R variants | 268    | 39            | 115       | 87            | 470           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
| Types of MC1R variants | 268    | 39            | 115       | 87            | 470           | 36.5                    |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |
|              |        |               |           |               |               |                         |                       |                           |

* The median ages at diagnosis of melanoma were estimated in melanoma patients carrying CDKN2A mutations from each continent and from all three continents for each category of MC1R variables (individual MC1R variants, any MC1R variant, number of MC1R variants, types of MC1R variants). NRHC = nonred hair color; RHC = red hair color.

† *P* values for the two-sided Jonckheere–Terpstra test used to test for a change in age at diagnosis of melanoma with presence of individual MC1R variants, presence of any variant or as the number and number and types of MC1R variants increased.

‡ Nonsynonymous MC1R variants that were not RHC variants.

§ Red hair color variants included R151C, R160W, D294H, and D84E.
The results in these subjects because a recent Swedish case-control study reported an increase in melanoma risk with an increase in the number of MC1R variants, and the risk was even higher in familial cases (43). Although the Italian CDKN2A mutation carriers in our study did not show an association between MC1R and melanoma risk, previous studies of unselected case patients and control subjects from different regions of Italy showed statistically significant associations between MC1R variants and melanoma risk (21,44,45). However, the results of our analysis in CDKN2A mutation carriers were also consistent with many previous case-control studies from various populations that showed an increased risk with multiple MC1R variants (21,43,46–49). An increase in melanoma risk with increase in the number of MC1R variants was also reported in a recent meta-analysis of the association between MC1R variants and melanoma risk in CDKN2A mutation carriers (50). It must be noted that the meta-analysis of published results was conducted on a much smaller sample size compared with the present analysis of raw genotypic and phenotypic data (96 vs 186 families) and did not address several points presented here, including the association of MC1R variants by type of CDKN2A mutation and the joint associations of MC1R variants and host phenotypes with melanoma risk.

When examining the number and types of MC1R variants, we observed that the increase in melanoma risk was generally higher in the presence of RHC variants. The classification of MC1R variants into RHC and NRHC was primarily based on the strength of association of these variants with red hair in populations of Celtic origin (17). This classification has been evolving over time and is not entirely uniform across studies investigating MC1R variants and the melanoma risk. We have chosen to restrict our categorization of RHC variants to the four MC1R variants consistently defined as RHC variants (R151C, R160W, D294H, and D84E).

The association of MC1R variants with melanoma risk may vary with the type of CDKN2A mutations. Indeed, a molecular basis for the link between CDKN2A and MC1R has been provided by in vitro studies that showed that the increased expression of p16INK4a after exposure to ultraviolet radiation is potentiated by α-melanocyte-stimulating hormone (α-MSH) through its binding to MC1R (51). In our analysis, the associations of MC1R variants with melanoma risk did not differ statistically significantly depending on whether CDKN2A mutations altered only the p16INK4a protein or both p16INK4a and p14ARF proteins.

It is not fully resolved whether the increased melanoma risk attributed to the MC1R variants is distinct from their association with pigmentation characteristics. Case-control studies have indicated that part of the association between melanoma risk and MC1R variants remains after stratification of phenotypic features suggesting that the association of MC1R is not exerted entirely through pigmentation (21,46–48). However, this issue has been scarcely investigated in CDKN2A mutation-positive families (15,16,21). This study showed that all four frequent MC1R variants, V60L, V92M, R151C, and R160W, were still associated with a statistically significantly increased melanoma risk, while adjusting for hair color or sunburn. Further stratified analysis based on hair color showed that the statistically significantly increase in risk was limited to subjects with brown or black hair, in agreement with a recent case-control study (52). These results strengthen the hypothesis that
MC1R variants may have a role in carcinogenesis in addition to its influence on pigment variation. Experimental (in vitro) studies have shown that, besides its role in pigmentation, α-MSH, which binds to MC1R, is involved in anti-apoptotic DNA repair and anti-inflammatory pathways (53–56). The additional association of hair color with melanoma risk is in agreement with results from genome-wide association studies that have identified several loci influencing pigmentation phenotypes (36,37). Our study also demonstrates that having high numbers of nevi was associated with a statistically significantly increased melanoma risk in \textit{CDKN2A} mutation carriers, independently of MC1R variants. A recent genome-wide association study (57), carried out by GenoMEL, in \textit{CDKN2A}-negative melanoma case patients and control subjects, identified independent associations of three loci with melanoma risk—16q24, encompassing \textit{MC1R}, 11q14–q21, encompassing the tyrosinase pigmentation gene (\textit{TYR}), and 9p21, adjacent to \textit{CDKN2A} and the methylthioadenosine phosphorylase (\textit{MTAP}) genes (\textit{CDKN2A}/\textit{MTAP}). The \textit{CDKN2A}/\textit{MTAP} locus was concurrently characterized as a nevus gene (58). Further investigation of the \textit{TYR} and \textit{CDKN2A}/\textit{MTAP} loci in families segregating \textit{CDKN2A} mutations will allow assessment of whether common variants of these genes also modify penetrance of \textit{CDKN2A} deleterious mutations.

Our analysis may have a few limitations. A slight decrease in age at melanoma diagnosis, which only reached statistical significance in the largest sample of European \textit{CDKN2A} mutation carriers and in mutation carriers from all three continents, was observed with the presence of RHC variants and as the number and types of variants increased. A decrease in age at melanoma diagnosis with increasing number of MC1R variants was previously reported to be mostly statistically significant in melanoma patients with multiple primary melanomas (15,22). Any information on the occurrence of single or multiple primary melanoma was not available for this analysis; however, we plan to investigate in the near future whether the association of MC1R with age at melanoma diagnosis differs in single vs multiple primary melanoma. The association of MC1R variants with melanoma risk was currently assessed by comparing affected and unaffected family members using an analytical method that accounts for the familial dependence and prevents inflation of the type I error rate (59). It should be noted that the odds ratios presented here are estimates of association in families similar to the sampled families and cannot be extrapolated to the general population. In all analyses, we used age at examination rather than age at diagnosis of melanoma because the host phenotypes that were examined jointly with MC1R variants vary over time and were measured at the time of examination. However, repeating the analyses using age at diagnosis of melanoma patients produced similar results for the association of MC1R variants and melanoma risk (data not shown).

In conclusion, this study shows that melanoma risk in \textit{CDKN2A} mutation carriers is modified by multiple factors that include MC1R variants, pigmentation, and nevus phenotypes. Investigation of other modifying genes, such as those identified by genome-wide association studies, may help clarify the complex mechanisms leading to familial melanoma. Such studies may have important consequences for improving melanoma risk assessment in families.

**Appendix**

The Melanoma Genetics Consortium (GenoMEL; http://www.genomel.org) included the following participating groups:

**Europe**

The participants of GenoMEL in Paris, France: Florence Demenais, Hamida Mohamdi, Valérie Chaudru, Eve Corda, Patricia Jeanm, and Eve Maubée (Inserm U946 and Université Paris Diderot, Fondation Jean Dausset—CEPH, Paris, France), Marie-Françoise Avril (AP-HP, Hôpital Cochin, Service de Dermatologie, Université Paris 5, Paris, France), Brigitte Bressac-de Paillerets, Fabienne Lesueur, and Mahaut de Lichy (Département de Génétique Moléculaire, Institut de Cancérologie Gustave Roussy, Villejuif, France).

The participants of GenoMEL in Emilia-Romagna, Italy: Maria Teresa Landi (Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD), Donata Calista, Giorgio Landi, Paola Minghetti, Daniela Capriossi, Pier Alberto Bertazzi, and Fabio Arcangeli (Dermatology Unit, Maurizio Bufalini Hospital, Cesena, Italy).

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References
1. Hussussian CJ, Struwing JP, Goldstein AM, et al. Germline p16 mutations in familial melanoma. Nat Genet. 1994;8(1):15–21.
2. Kamb A, Shattuck-Eidens D, Eeles RA, et al. Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. Nat Genet. 1994;8(1):23–26.
3. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993; 366(6456):704–707.
4. Serrano M, Gomez-Lahoz E, DePinho RA, et al. The Ink4a tumor suppressor gene family. Science. 1995;267(5195):249–252.
5. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell. 1998;92(6):725–734.
6. Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell. 1998;92(6):713–723.
7. Goldstein AM, Chan M, Harland M, et al. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. Cancer Res. 2006;66(20):9818–9828.
8. Bishop DT, Demenais F, Goldstein AM, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. J Natl Cancer Inst. 2002;94(12):894–903.
9. Goldstein AM, Martinez M, Tucker MA, Demenais F. Gene-covariate interaction between dysplastic nevi and the CDKN2A gene in American melanoma-prone families. Cancer Epidemiol Biomarkers Prev. 2000;9(9): 889–894.
10. Chaudru V, Chompret A, Bresac-de Paillerets B, Spatz A, Avril MF, Demenais F. Influence of genes, nevi, and sun-sensitivity on melanoma risk in a family sample unscreened by family history and in melanoma-prone families. J Natl Cancer Inst. 2004;96(10):785–795.
11. Busca R, Ballotti R. Cyclic AMP as a key messenger in the regulation of skin pigmentation. Pigment Cell Res. 2004;17(2):60–69.
12. Raimondi S, Seri F, Gandini S, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. Int J Cancer. 2008;122(2):273–2760.
13. Box NF, Duffy DL, Chen W, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. Am J Hum Genet. 2001;69(4):765–773.
14. van der Velden PA, Sandkuijl LA, Bergman W, et al. Melanocortin-1 receptor variant Arg151Cys modifies melanoma risk in Dutch families with melanoma. Am J Hum Genet. 2001;69(4):774–779.
15. Goldstein AM, Landi MT, Tsang S, Fraser MC, Munroe DJ, Tucker MA. Association of MC1R variants and risk of melanoma in melanoma-prone families with CDKN2A mutations. Cancer Epidemiol Biomarkers Prev. 2005;14(9):2208–2212.
16. Chaudru V, Llaud K, Avril MF, et al. Melanocortin-1 receptor (MC1R) gene variants and dysplastic nevi modify penetrance of CDKN2A muta-
38. Garcia-Borrón JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res. 2005;18(6):393–410.

39. Beaumont KA, Shekar SN, Newton RA, et al. Receptor function, dominant negative activity and phenotype correlations for MCIR variant alleles. Hum Mol Genet. 2007;16(18):2249–2260.

40. Ringholm A, Klovins J, Rudzish R, Phillips S, Rees JL, Schiöth HB. Pharmacological characterization of loss of function mutations of the human melanocortin 1 receptor that are associated with red hair. J Invest Dermatol. 2004;123(5):917–923.

41. Xu X, Thörnwall M, Lundin LG, Chhajlani V. Val92Met variant of the melanocyte stimulating hormone receptor gene. Nat Genet. 1996;14(4):384.

42. Sanchez-Laorden BL, Jimenez-Cervantes C, Garcia-Borrón JC. Regulation of human melanocortin 1 receptor signaling and trafficking by Thr-308 and Ser-316 and its alteration in variant alleles associated with red hair and skin cancer. J Biol Chem. 2007;282(5):3241–3251.

43. Hoioni V, Tuominen R, Käller M, et al. MCIR variation and melanoma risk in the Swedish population in relation to clinical and pathological parameters. Pigment Cell Melanoma Res. 2009;22(2):196–204.

44. Pastorio L, Casano R, Bruno W, et al. Novel MCIR variants in Ligurian melanoma patients and controls. Hum Mutat. 2004;24(1):103.

45. Fargnoli MC, Spica T, Sera F, et al. Re: MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. J Natl Cancer Inst. 2006;98(2):144–145; author reply 145–146.

46. Palmer JS, Duffy DL, Box NF, et al. Melanocortin-1 receptor polymorphism and risk of melanoma: is the association explained solely by pigment phenotype? Am J Hum Genet. 2000;66(1):176–186.

47. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor structure and functional regulation. Melanocortin-1 receptor signaling markedly induces the expression of the human melanocyte stimulating hormone receptor gene. Hum Mol Genet. 2005;14(6):1413–1420.

48. Pavey S, Gabrielli B. Alpha-melanocyte stimulating hormone potentiates p16/CDKN2A expression in human skin after ultraviolet irradiation. Cancer Res. 2002;62(3):875–880.

49. Kanetsky P, Panossian S, Elder DE, et al. Anti-inflammatory and anti-invasive effects of alpha-melanocyte-stimulating hormone in human melanoma cells. Br J Cancer. 2003;89(10):2004–2015.

50. Smith AG, Luk N, Newton RA, Roberts DW, Sturm RA, Muscat GE. Melanocortin-1 receptor signaling markedly induces the expression of the N-RAS4A nuclear receptor subgroup in melanocytic cells. J Biol Chem. 2005;280(18):15264–15270.

51. Hauser JE, Kadekaro AL, Vananghand A, et al. Melanin content and MCIR function independently affect UV-induced DNA damage in cultured human melanocytes. Pigment Cell Res. 2008;21(4):303–314.

52. Eves P, Haycock J, Layton C, et al. Anti-inflammatory and anti-invasive effects of alpha-melanocyte-stimulating hormone in human melanoma cells. Br J Cancer. 2008;100(14):892–905.

53. Smith AG, Luk N, Newton RA, Roberts DW, Sturm RA, Muscat GE. Melanocortin-1 receptor signaling markedly induces the expression of the N-RAS4A nuclear receptor subgroup in melanocytic cells. J Biol Chem. 2008;283(18):12564–12570.

54. Bishop DT, Demenais F, Isles MM, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet. 2009;41(8):920–925.

55. Falchi M, Bataille V, Hayward NK, et al. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nat Genet. 2009;41(8):915–919.

56. Slager SL, Schaid DJ. Evaluation of candidate genes in case-control studies: a statistical method to account for related subjects. Am J Hum Genet. 2001;68(6):1457–1462.

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