

Population-Based Study of Natural Variation in the *Melanocortin-1 Receptor* Gene and Melanoma

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Abstract

Natural variation in the coding region of the *melanocortin-1 receptor (MC1R)* gene is associated with constitutive pigmentation phenotypes and development of melanoma and non-melanoma skin cancers. We investigated the effect of *MC1R* variants on melanoma using a large, international population-based study design with complete determination of all *MC1R* coding region variants. Direct sequencing was completed for 2,202 subjects with a single primary melanoma (controls) and 1,099 subjects with second or higher-order primary melanomas (cases) from Australia, the United States, Canada, and Italy. We observed 85 different *MC1R* variants, 10 of which occurred at a frequency >1%. Compared with controls, cases were more likely to carry two previously identified red hair ("R") variants [D84E, R151C, R160W, and D294H; odds ratio (OR), 1.6; 95% confidence interval (95% CI), 1.1-2.2]. This effect was similar among individuals carrying one R variant and one r variant (defined as any non-R *MC1R* variant; OR, 1.6; 95% CI, 1.3-2.2) and among those carrying only one R variant (OR, 1.5; 95% CI, 1.1-1.9). There was no statistically significant association among those carrying only one or two r variants. Effects were similar across geographic regions and categories of pigmentation characteristics or number of moles. Our results confirm that *MC1R* is a low-penetrance susceptibility locus for melanoma, show that pigmentation characteristics may not modify the relationship of *MC1R* variants and melanoma risk, and suggest that associations may be smaller than previously reported in part due to the study design. (Cancer Res 2006; 66(18): 9330-7)

Introduction

Since the first publication of DNA sequence variation in the *melanocortin-1 receptor (MC1R)* gene (MIM 155555) and human

hair color and skin type (1), numerous studies have investigated the relationship between *MC1R* variants and cutaneous pigmentation characteristics (reviewed in ref. 2). Studies also have focused on the relationship of *MC1R* with development of malignant melanoma (3-8). Results from these studies have shown that *MC1R* variants and particularly those variants most strongly associated with red hair color (termed R variants, including D84E, R151C, R160W, and D294H; see ref. 9) increase risk of melanoma.

Further evidence of the role of *MC1R* variants in melanoma etiology comes from studies of melanoma-prone families that show a younger average age of onset of melanoma in families inheriting mutations in both *MC1R* and *cyclin-dependent kinase inhibitor 2A (CDKN2A)* than in families with mutations in *CDKN2A* alone (10-13).

In this study, we examine the frequencies of *MC1R* variants and their associations with melanoma in a large population-based investigation. The study involved population-based recruitment of patients with an incident second primary melanoma or a higher-order primary melanoma (i.e., third, fourth, etc.). The "control" group represents the population at risk for these subsequent primary melanomas (i.e., patients who are diagnosed with a first primary melanoma). This design enriches the sampling for rare variants that are associated with the disease (14). The validity of the design for evaluating risk factors for melanoma has been examined in detail in a recent article (15). By sequencing the entire *MC1R* coding region in this large population-based sample, the study provides the most comprehensive data on *MC1R* in melanoma to date.

Materials and Methods

Setting and population. From January 1, 1998 to August 31, 2003, individuals with a newly diagnosed melanoma were eligible to participate in a large, international study exploring the genetic and environmental epidemiology of melanoma. Individuals with a diagnosis of incident first invasive melanoma (controls) and incident second or higher-order invasive or *in situ* melanoma (cases) were identified from eight population-based cancer registries in Australia (New South Wales and Tasmania), Canada (British Columbia and Ontario), Italy (Turin), and the United States (Orange County, California; North Carolina; and New Jersey) and one melanoma clinic in Michigan that sees a large fraction of patients within this region. Each participant signed informed consent that had been approved

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Table 1. Demographic and phenotypic variables by geography

Risk factor	Total (N = 3,218), n (%)	North America (n = 1,899), n (%)	Australia (n = 1,179), n (%)	Italy (n = 140), n (%)
Age				
<30	145 (5)	92 (5)	47 (4)	6 (4)
30-49	834 (26)	566 (30)	215 (18)	53 (38)
50-69	1,314 (41)	776 (41)	474 (40)	64 (46)
≥70	925 (29)	465 (24)	443 (38)	17 (12)
Sex				
Male	1,773 (55)	1,006 (53)	698 (59)	69 (49)
Female	1,445 (45)	893 (47)	481 (41)	71 (51)
Phenotypic index				
1	253 (8)	175 (10)	64 (5)	14 (10)
2	610 (20)	371 (21)	199 (17)	40 (29)
3	1,211 (39)	688 (39)	472 (40)	51 (37)
4	838 (27)	454 (25)	355 (30)	29 (21)
5	187 (6)	97 (5)	85 (7)	5 (4)
Mole count on back				
<5	1,081 (35)	556 (30)	476 (40)	49 (39)
5-10	692 (22)	389 (21)	277 (24)	26 (21)
11-25	703 (22)	432 (24)	248 (21)	23 (19)
>26	666 (21)	467 (25)	173 (15)	26 (21)

by the Institutional Review Board or similar committee that oversees human research at each participating institution. Participants completed study requirements that included a brief self-administered questionnaire to provide pigmentation characteristics and lifetime residential history, an extensive telephone-administered questionnaire eliciting information on demographic variables, and skin sensitivity and sun exposure history, and each gave one or more buccal swabs (94%) or a blood sample (6%) for isolation of genomic DNA. All biosamples were shipped to the coordinating center (Memorial Sloan-Kettering Cancer Center, New York, NY). Methodologic details of this study, including duration and timing of ascertainment for case and control subjects for each center, are described in Begg et al. (16).

MC1R genotyping. All *MC1R* genotypes were determined at a single center as previously described in Kanetsky et al. (17). Genomic DNA was isolated from buccal cells using either a modified version of the Richards' protocol (18) or by Puregene DNA Purification kits for buccal cells (Gentra Systems, Minneapolis, MN). DNA was isolated from blood lymphocytes using Qiagen QIAamp DNA Blood kit (Qiagen, Inc., Valencia, CA). Using a MJ Research (Waltham, MA) PTC-100 thermocycler, standard PCR technique was used to amplify the entire 951-nucleotide *MC1R* coding region. All amplified products were directly sequenced on an ABI Prism 377 or 3100 (Applied Biosystems, Foster City, CA) using BigDye Terminators (Applied Biosystems) according to the manufacturer's specifications. Sequencing primers consisted of either a set of two or four oligonucleotides: 5'-GCCATGAGCACCAGCATAG-3' and 5'-GACCACACAAATATCACCACCT or 5'-TCGTCTTCAGCAGCTCTTC-3', 5'-TTTAAGGCCAAGCCCTGGT-3', 5'-AACCTGCACTCACCATGTA-3', and 5'-CTGCAGGTGATCAGTCAAT, respectively. *MC1R* chromatograms were read with the aid of Sequencher software version 4.0.5 (Gene Codes Corp., Ann Arbor, MI) and/or SeqScape software version 1.0 to 2.1.1 (Applied Biosystems). A minority of novel variants observed only once were verified independently. All *MC1R* genotypes were double entered into a customized Microsoft Office Access 2003 database before delivery to the coordinating center for statistical analysis.

Data coding. Information obtained from the self-administered questionnaire instrument (19) was used to create a phenotypic index that was based on participant responses to hair color (black or dark brown hair = 1; light brown or blond hair = 2; red hair = 3), eye color (black or brown eyes = 0; hazel, green, gray, or blue eyes = 1), and inability to tan in response to sun

exposure (no = 0; yes = 1). An index score of 1 or 2 signifies overall darker cutaneous phenotype and indicates a low phenotypic risk, an index score of 3 indicates medium phenotypic risk, and an index score of 4 or 5 signifies overall fairer cutaneous phenotype and indicates high phenotypic risk. Using a glossy colored guide to aid in differentiating between nevi and other skin lesions, subjects were asked to have the nevi on their backs counted by a family member or friend; the number of self-reported nevi on the back was categorized as <5, 5 to 10, 11 to 25, and >26.

We calculated allele frequencies for the *MC1R* consensus sequence (Genbank accession no. AF326275) and observed *MC1R* variants and used the nomenclature and definitions suggested by Sturm et al. (9) to group *MC1R* variants as higher-risk R variants (D84E, R151C, R160W, and D294H) or lower-risk r variants (all other variants excluding synonymous changes), acknowledging that these risk categories are inexact because the precise functional status of many *MC1R* variants is unknown. We also categorized *MC1R* R and r variants using two alternative coding schemes: the first additionally included the R142H and the g.86_87insA variant (along with the other four observed insertion/deletion variants) as R variants based on prior work, showing the importance of these variants in pigmentation or *MC1R* function (5, 20, 21), and the second used previously published results from a Sorting Intolerant from Tolerant analysis, which identified *MC1R* protein positions that are predicted intolerant to amino acid substitutions, thus indicating those variants expected to be higher-risk based on putative functional importance (17).

Statistical analysis. Contingency table analysis was used to compare univariate associations [odds ratios (OR)] of individual or categories of *MC1R* variants and case/control status. Unconditional logistic regression was used to obtain adjusted estimates. These models were adjusted for age of diagnosis of most recent melanoma (continuous variable), sex, study center (eight indicator variables), and a term for the interaction of age and sex. Adjusted ORs (aOR) and corresponding 95% confidence intervals (95% CI) are presented.

Results

Study ascertainment. A total of 6,868 potentially eligible patients diagnosed with melanoma during the designated accrual period were ascertained. After physician and patient refusals and other exclusions, a total of 3,584 individuals (52% overall

participation rate) with adequate specimens for genetic analysis were available. These included 2,470 first primary melanoma controls and 1,210 multiple primary melanoma cases; 96 controls became cases during the course of the study and thus met eligibility criteria for both groups and were included once as a melanoma case and once as a melanoma control in analyses. The distribution of age, sex, phenotypic index, and mole count on back for the total study sample and for individual geographic regions is given in Table 1. Patient recruitment as well as the comparison of demographic,

phenotypic, and tumor characteristics between case and control subject have been published previously (15, 22). DNA samples from 3,218 (90%) of these individuals, including 2,202 (89%) controls and 1,099 (91%) cases (of which 83 were eligible as both a case and a control), were successfully genotyped at the *MC1R* locus.

MC1R variant determination. *MC1R* variants and their observed allele frequencies by case status are given in Table 2. In total, we detected 85 unique *MC1R* variants; 60 corresponded to nonsynonymous amino acid substitutions, 20 resulted in

Table 2. Allele frequency of *MC1R* variants in individuals with first primary (controls) and multiple primary (cases) melanoma

Nucleotide change	Amino acid change	Control (no. chromosomes = 4,404),* 2n (%)	Case (no. chromosomes = 2,198),* 2n (%)
Consensus	None	1,487 (33.8)	653 (29.7)
Nonsynonymous			
g.178T>G	V60L	584 (13.3)	288 (13.1)
g.252C>A	D84E	78 (1.8)	58 (2.6)
g.274G>A	V92M	439 (10.0)	222 (10.1)
g.425G>A	R142H	43 (1.0)	23 (1.0)
g.451C>T	R151C	582 (13.2)	349 (15.9)
g.464T>C	I155T	67 (1.5)	30 (1.4)
g.478C>T	R160W	432 (9.8)	237 (10.8)
g.488G>A	R163Q	184 (4.2)	109 (5.0)
g.880G>C	D294H	122 (2.8)	78 (3.5)
Rare [†]	†	72 (1.6)	36 (1.6)
Insertion/deletion [‡]	‡	40 (0.9)	17 (0.8)
Synonymous			
g.942A>G	T314T	541 (12.3)	270 (12.3)
Rare [§]	§	38 (0.9)	16 (0.7)
Any variant			
0		688 (15.6)	272 (12.4)
1		1,598 (36.3)	762 (34.7)
2		1,528 (34.7)	820 (37.3)
3		510 (11.6)	312 (14.2)
4		80 (1.8)	32 (1.5)
Any nonsynonymous or insertion/deletion variant			
0		750 (17.0)	300 (13.6)
1		1,968 (44.7)	908 (41.3)
2		1,680 (38.1)	984 (44.8)
3		6 (0.1)	6 (0.3)

*Number of individuals genotyped is half the number of chromosomes genotyped.

† A group indicating carriage of any of the following 51 nonsynonymous SNPs (previously unpublished variants are noted in bold): **g.92C>T (T31I; n = 1)**; **g.104G>A (C35Y; n = 1)**; **g.106C>G (L36V; n = 1)**; **g.112G>A (V38M; n = 8)**; **g.133T>C (F45L; n = 4)**; **g.190G>A (A64T; n = 1)**; **g.200G>A (R67Q; n = 1)**; **g.241G>C (A81P; n = 1)**; **g.247T>C (S83P; n = 7)**; **g.284C>T (T95M; n = 2)**; **g.292A>G (I98V; n = 1)**; **g.310G>A (G104S; n = 1)**; **g.334G>A (V112M; n = 1)**; **g.350A>T (D117V; n = 1)**; **g.359T>C (I120T; n = 1)**; **g.364G>A (V122M; n = 5)**; **g.373T>C (C125R; n = 1)**; **g.383T>A (M128K; n = 1)**; **g.392G>A (S131N; n = 1)**; **g.434C>T (S145F; n = 1)**; **g.445G>A (A149T; n = 1)**; **g.456C>A (Y152X; n = 9)**; **g.466C>G (V156L; n = 1)**; **g.467T>C (V156A; n = 2)**; **g.479G>A (R160Q; n = 2)**; **g.482C>T (A161V; n = 1)**; **g.487C>T (R163X; n = 1)**; **g.504C>G (I168M; n = 2)**; **g.515G>T (S172I; n = 1)**; **g.547T>G (Y183D; n = 1)**; **g.550G>A (D184N; n = 1)**; **g.583T>G (F195V; n = 2)**; **g.637C>T (R213W; n = 7)**; **g.652G>A (A218T; n = 6)**; **g.653C>G (A218G; n = 1)**; **g.667C>T (R223W; n = 2)**; **g.725C>T (T242I; n = 2)**; **g.743G>T (G248V; n = 1)**; **g.766C>T (P256S; n = 2)**; **g.814A>G (T272A; n = 1)**; **g.815C>A (T272K; n = 1)**; **g.823T>C (C275R; n = 1)**; **g.832A>G (K278E; n = 3)**; **g.837C>A (N279K; n = 2)**; **g.860T>G (I287S; n = 2)**; **g.861C>G (I287M; n = 2)**; **g.865T>C (C289R; n = 1)**; **g.869A>G (N290S; n = 1)**; **g.895G>A (A299T; n = 1)**; **g.900C>G (F300L; n = 1)**; **g.917G>A (R306H; n = 4)**.

‡ A group indicating carriage of any of the following five insertion/deletion variants (previously unpublished variants are noted in bold): **g.86_87insA** (frameshift; premature termination at codon 42; n = 42); **g.208delC** (frameshift; premature termination at codon 87; n = 1); **g.537_538insC** (frameshift; premature termination at codon 238; n = 9); **g.581_583delITCT** (deletion of a F residue at position 195; n = 1); **g.637_655del** (frameshift; premature termination at codon 307; n = 2).

§ A group indicating carriage of any of the following 19 synonymous SNPs (previously unpublished variants are noted in bold): **g.102G>C (R34R; n = 1)**; **g.159G>A (L53L; n = 1)**; **g.285G>A (T95T; n = 1)**; **g.333G>A (A111A; n = 1)**; **g.357C>A (V119V; n = 1)**; **g.399C>T (C133C; n = 5)**; **g.408C>G (G136G; n = 1)**; **g.417C>G (A139A; n = 1)**; **g.453C>G (R151R; n = 6)**; **g.492C>T (A164A; n = 5)**; **g.546C>T (Y182Y; n = 1)**; **g.555C>T (H185H; n = 1)**; **g.699G>A (Q233Q; n = 9)**; **g.717C>T (G239G; n = 2)**; **g.765C>A (G255G; n = 1)**; **g.792C>T (I264I; n = 4)**; **g.819C>T (C273C; n = 1)**; **g.876C>T (I292I; n = 1)**; **g.948C>T (S316S; n = 11)**.

Table 3. Association of *MC1R* variants and melanoma: crude, adjusted, and by geography

Variant	Control, <i>n</i> (%)	Case, <i>n</i> (%)	OR (95% CI)	aOR* (95% CI)	North America (<i>n</i> = 1,179)	Australia (<i>n</i> = 1,899)	Italy (<i>n</i> = 140)
V60L							
V/V	1,658 (75)	838 (76)	1.00	1.00	1.00	1.00	1.00
V/L	504 (23)	234 (21)	0.95 (0.80-1.1)	0.99 (0.82-1.2)	0.96 (0.75-1.2)	0.98 (0.74-1.3)	1.5 (0.46-4.6)
L/L	40 (2)	27 (2)					
D84E							
D/D	2,125 (97)	1,042 (95)	1.00	1.00	1.00	1.00	1.00
D/E	76 (3)	56 (5)	1.5 (1.1-2.1)	1.4 (0.95-2.1)	1.4 (0.82-2.4)	1.3 (0.73-2.2)	1.8 (0.16-20)
E/E	1 (<1)	1 (<1)					
V92M							
V/V	1,795 (82)	889 (81)	1.00	1.00	1.00	1.00	1.00
V/M	375 (17)	198 (18)	1.0 (0.87-1.3)	0.94 (0.77-1.2)	0.98 (0.74-1.3)	0.92 (0.69-1.2)	0.95 (0.19-4.8)
M/M	32 (1)	12 (1)					
R142H							
R/R	2,159 (98)	1,076 (98)	1.00	1.00	1.00	1.00	1.00
R/H	43 (2)	23 (2)	1.1 (0.64-1.8)	1.4 (0.79-2.4)	1.7 (0.92-3.3)	1.0 (0.35-2.9)	NE
H/H	0 (0)	0 (0)					
R151C							
R/R	1,654 (75)	776 (71)	1.00	1.00	1.00	1.00	1.00
R/C	514 (23)	297 (27)	1.3 (1.1-1.5)	1.3 (1.1-1.6)	1.1 (0.90-1.5)	1.4 (1.1-1.9)	0.80 (0.16-4.0)
C/C	34 (2)	26 (2)					
I155T							
I/I	2,135 (97)	1,069 (97)	1.00	1.00	1.00	1.00	1.00
I/T	67 (3)	30 (3)	0.89 (0.58-1.4)	0.81 (0.50-1.3)	0.95 (0.52-1.7)	0.71 (0.34-1.5)	NE
T/T	0 (0)	0 (0)					
R160W							
R/R	1,789 (81)	871 (79)	1.00	1.00	1.00	1.00	1.00
R/W	394 (18)	219 (20)	1.1 (0.95-1.4)	1.2 (0.98-1.5)	1.3 (1.0-1.7)	1.0 (0.75-1.4)	1.4 (0.27-6.8)
W/W	19 (<1)	9 (<1)					
R163Q							
R/R	1,996 (91)	993 (90)	1.00	1.00	1.00	1.00	1.00
R/Q	198 (9)	103 (9)	1.0 (0.81-1.3)	1.1 (0.81-1.4)	1.0 (0.73-1.5)	1.2 (0.76-1.8)	NE
Q/Q	8 (<1)	3 (<1)					
D294H							
D/D	2,085 (95)	1,024 (93)	1.00	1.00	1.00	1.00	1.00
D/H	112 (5)	72 (7)	1.3 (0.97-1.8)	1.4 (1.0-1.9)	1.2 (0.74-1.9)	1.5 (0.91-2.4)	2.6 (0.21-32)
H/H	5 (<1)	3 (<1)					
Rare [†] nonsynonymous single nucleotide polymorphism (SNP)							
con [‡] /con	2,131 (97)	1,065 (97)	1.00	1.00	1.00	1.00	1.00
con/rare	70 (3)	32 (3)	0.96 (0.63-1.5)	0.94 (0.60-1.5)	0.83 (0.43-1.6)	1.3 (0.63-2.5)	1.1 (0.13, 10)
rare/rare	1 (<1)	2 (<1)					
Insertion/deletion							
con/con	2,163 (98)	1,082 (98)	1.00	1.00	1.00	1.00	1.00
con/var	38 (2)	17 (2)	0.87 (0.49-1.6)	0.96 (0.51-1.8)	0.23 (0.05-1.0)	1.9 (0.80-4.6)	NE [§]
var/var	1 (<1)	0 (0)					
r variants							
0	1,002 (46)	509 (46)	1.00	1.00	1.00	1.00	1.00
1	942 (43)	457 (42)	0.98 (0.82-1.1)	0.93 (0.79-1.1)	1.0 (0.78-1.3)	0.88 (0.70-1.1)	1.1 (0.38-3.1)
2	258 (12)	133 (12)	1.0 (0.80-1.3)	1.0 (0.79-1.3)	1.1 (0.71-1.5)	1.0 (0.74-1.4)	NE
R [¶] variants							
0	1,178 (54)	486 (44)	1.00	1.00	1.00	1.00	1.00
1	835 (38)	504 (46)	1.5 (1.3-1.7)	1.5 (1.3-1.8)	1.5 (1.1-1.9)	1.5 (1.2-1.9)	1.0 (0.30-3.5)
2	189 (9)	109 (10)	1.4 (1.1-1.8)	1.5 (1.2-2.0)	1.5 (0.97-2.3)	1.4 (0.97-2.1)	2.3 (0.20-26)
Aggregate							
con/con	375 (17)	150 (14)	1.00	1.00	1.00	1.00	1.00
con/r	545 (25)	204 (19)	0.94 (0.73-1.2)	0.93 (0.71-1.2)	0.89 (0.61-1.3)	0.95 (0.63-1.4)	0.71 (0.20-2.5)

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Table 3. Association of *MC1R* variants and melanoma: crude, adjusted, and by geography (Cont'd)

Variant	Control, n (%)	Case, n (%)	OR (95% CI)	aOR* (95% CI)	North America (n = 1,179)	Australia (n = 1,899)	Italy (n = 140)
r/r	258 (12)	132 (12)	1.3 (0.96-1.7)	1.3 (0.98-1.8)	1.4 (0.90-2.1)	1.3 (0.81-2.1)	NE
con/R	439 (20)	250 (23)	1.4 (1.1-1.8)	1.5 (1.1-1.9)	1.5 (1.1-2.2)	1.4 (0.90-2.0)	0.30 (0.03-2.7)
R/r	396 (18)	254 (23)	1.6 (1.3-2.1)	1.6 (1.3-2.2)	1.6 (1.1-2.3)	1.7 (1.1-2.5)	1.7 (0.37-7.8)
R/R	189 (9)	109 (10)	1.4 (1.1-2.0)	1.6 (1.1-2.2)	1.5 (0.94-2.3)	1.5 (0.94-2.5)	1.8 (0.15-22)

NOTE: For all individual variants, the ORs represent the comparison of carriage of at least one variant to carriage of no variants (i.e., var/* versus con/con).

*ORs are adjusted for age at most recent melanoma diagnosis, sex, age-sex interaction, and center.

†A group indicating carriage of at least 1 of 52 nonsynonymous SNP with a MAF <1% (see Table 1 footnote).

‡Consensus *MC1R* sequence.

§OR is not estimable.

||Carriage of V60L, V92M, R142H, I155T, R163Q, any rare nonsynonymous, or any insertion/deletion variant in the absence of an R variant (*).

¶Carriage of D84E, R151C, R160W, or D294H.

synonymous amino acid changes, and 5 were insertion/deletions. Nine nonsynonymous (V60L, D84E, V92M, R142H, R151C, I155T, R160W, R163Q, and D294H) and one synonymous (T314T) variants were observed at a minor allele frequency (MAF) >1%. To the best of our knowledge, 29 rare nonsynonymous, 14 rare synonymous, and 3 insertion/deletion variants, including a 17-bp deletion, are novel and have not yet been reported in the published literature (see Table 2 footnotes).

Similar to other reports (23, 24), the occurrence of a variant in the first 50 codons of *MC1R* was rare in our data. Thus, we allowed an individual to be missing up to 150 nucleotides at the 5'-end of the *MC1R* coding region due to weak sequence signal, which occurred in 698 (22%) sequenced individuals. We chose this cut point so that, for each sample, it was possible to detect the commonly occurring V60L (g.178T>G) variant. Based on the number of observed rare variants ($n = 59$) in the first 150 nucleotides of the remaining 2,520 (78%) individuals, we calculated that we missed detection of, at maximum, only 16 rare variants because of failed *MC1R* sequence at the extreme 5'-end of the coding region.

There were three instances of an ambiguous translation from the nucleotide change to corresponding amino acid designation because three individuals carried two variants that fell within the same codon. Although it is not possible with genotyping data alone to determine the phase of these nucleotide changes (i.e., whether they occur in *cis* or *trans* orientation), analysis of inferred *MC1R* haplotypes in our study population indicates that, with few exceptions, *MC1R* variants are inherited individually on an otherwise genetic background of the consensus sequence (results not presented). Therefore, we assumed that these nucleotide changes were in *trans* orientation and assigned amino acid changes accordingly. The first individual carried the R151C (g.451C>T) and R151R (g.453C>G) variants, the second carried the R160W (g.478C>T) and R160Q (g.479G>A) variants, and the third carried the T272A (g.814A>G) and T272K (g.815C>A) variants.

Overall, 86% of individuals carried at least one *MC1R* variant; 84% of all individuals carried nonsynonymous and insertion/deletion variants. Carriage of synonymous variants alone is an uncommon event. One thousand four hundred ten individuals (43.8%) carried one, 1,288 (40.0%) carried two, and 6 (0.19%) carried three nonsynonymous or insertion/deletion variants.

***MC1R* variants and melanoma.** Table 3 shows the crude and aOR for associations between *MC1R* variants and melanoma. Despite our large sample size, there were few homozygous carriers of individual variants or compound heterozygous carriers with rare variants. Hence, for variant-specific associations, we present the OR for carriage of at least one variant versus homozygous carriage of the *MC1R* consensus. There was no association between the V60L, V92M, I155T, and R163Q variants and case status as evident by aORs near 1.0 and nonsignificant 95% CIs. In contrast, cases were more likely to carry the R variants and the R142H variant compared with controls as indicated by modest aORs in the range of 1.2 to 1.4 and statistically or borderline significant 95% CIs. In aggregate, case status was not associated with carriage of nonsynonymous variants that occur at a MAF <0.01 (aOR, 0.94; 95% CI, 0.60-1.5) nor with carriage of insertion/deletion variants (aOR, 0.96; 95% CI, 0.51-1.8).

Taking into account both the total number and risk group (i.e., R or r) of *MC1R* variants, there was a statistically significant trend ($P < 0.001$) toward carriage of multiple and higher-risk variants among cases compared with controls. Compared with individuals who carry two copies of the *MC1R* consensus sequence (con/con), case status was not associated with carriage of one r (con/r; aOR, 0.93; 95% CI, 0.71-1.2) or two or more r variants in the absence of an R variant (r/r; aOR, 1.3; 95% CI, 0.98-1.8). In contrast, cases were more likely to carry only one R variant (con/R; aOR, 1.5; 95% CI, 1.1-1.9) or one R variant in combination with a second (or more) variant regardless of its risk category (R/r; aOR, 1.6; 95% CI, 1.3-2.2; R/R; aOR, 1.6; 95% CI, 1.1-2.2). We obtained similar results for analyses that used the two alternative *MC1R* coding schemes (results not shown) that excluded the 83 controls who later became cases (results not shown) and that excluded cases with a second or higher-order diagnosis of melanoma *in situ* (Table 4).

aORs for carriage of *MC1R* variants were more similar between individuals recruited from Australian (59% of participants) and North American (37% of participants) study centers compared with those recruited through Turin, Italy (4% of participants; see Table 3). Although it seems that estimated ORs for some specific *MC1R* variants, including V60L, D84E, R160W, and D294H, may be stronger among Italians, interpretation is guarded because these associations did not reach statistical significance and this center

Table 4. Association of *MC1R* variants and melanoma when multiple primary cases are limited to invasive melanoma only

<i>MC1R</i> genotype	aOR* (95% CI)	
	All patients (n = 3,218)	Invasive only (n = 2,850)
con/con	1.00	1.00
con/r	0.93 (0.71-1.2)	0.93 (0.68-1.3)
r/r	1.3 (0.98-1.8)	1.4 (0.98-2.0)
con/R	1.5 (1.1-1.9)	1.4 (0.99-1.9)
R/r	1.6 (1.3-2.2)	1.8 (1.4-2.5)
R/R	1.6 (1.1-2.2)	1.6 (1.1-2.3)

*ORs are adjusted for age at most recent melanoma diagnosis, sex, age-sex interaction, and center.

had a small sample size. Associations of melanoma and *MC1R* risk groups were similar across the three geographic regions.

MC1R and melanoma by levels of pigmentation and nevi.

We did stratified analysis of *MC1R*-melanoma associations based on categories of phenotypic risk (low, medium, and high) as determined from the phenotypic index. With few exceptions, most stratum-specific associations did not reach statistical significance (Fig. 1A). Comparing the three phenotypic index groups, the pattern of aORs across *MC1R* genotype categories seemed similar.

We also determined associations of *MC1R* genotype with melanoma among categories of self-reported mole count on the back (<5, 5-10, 11-25, and >25; Fig. 1B). Among individuals with five or fewer moles, an elevated aOR was noted among those with con/r genotypes compared with those with con/con genotypes (aOR, 1.6; 95% CI, 0.93-2.6). aORs became increasingly stronger and statistically significant with corresponding increases in *MC1R* risk genotype, reaching an aOR of 2.8 and 2.7 for the R/r and R/R genotype groups, respectively. Although associations were weaker and did not reach statistical significance, a similar pattern of increasing aORs across *MC1R* genotypes was noted among individuals with >25 and 5 to 10 moles. No obvious pattern of risk was evident among individuals reporting 11 to 25 moles.

Discussion

We show that carriage of *MC1R* variants is associated with melanoma status and that risk is largely driven by carriage of the R variants (i.e., D84E, R151C, R160W, and D294H) as well as the number and combination of variants present. The findings from our study confirm that *MC1R* is a low-penetrance gene for melanoma and are consistent, although somewhat attenuated, with previously published studies (4, 5, 7, 8).

This study represents by far the largest investigation of *MC1R* variants and melanoma to date. Because all participants in this study have melanoma, the observed *MC1R* allele frequencies are higher than previous reports among populations of healthy individuals yet are generally consistent with allele frequencies reported among other melanoma case groups (5, 7). We detected 46 previously unreported variants in *MC1R*; all were rare. Although we had a very large sample size, we had small numbers of rare

variants. Thus, their contribution to melanoma risk was difficult to evaluate and is unlikely to be large.

Although our associations between *MC1R* variants and melanoma are consistent with previous studies, there are notable differences in the strengths of association observed in our study compared with those observed by other investigators. In most previously published work, associations between *MC1R* variants and melanoma are stronger than those found in our study. Palmer et al. (4) reported a 2.2- and 4.1-fold odds of melanoma among Australians who carry one or two copies of the R151C, R160W, and D294H variants. ORs reported by Kennedy et al. (5) among Dutch study participants were 3.1 and 4.9 for carriage of one or two copies, respectively, of any *MC1R* variant. A recent report among a French clinical population noted strong association between melanoma and carriage of one (OR, 4.3) or two (OR, 6.8) *MC1R* variants (7), whereas that from an Italian sample found that melanoma cases were over twice as likely to carry at least one *MC1R* and nearly four times more likely to carry two variants (8).

There are several methodologic differences between our study and other published investigations that may explain, in part, the difference in the observed strengths of association. Three studies used clinic- and/or hospital-based recruitment of melanoma cases for which individuals with early-stage melanoma may have been underrepresented (5, 7, 8). Indeed, a recent finding by Landi et al. (8) showed that the R151C, R160W, and D294H variants were more common among individuals with thicker melanomas (>1.06 mm). Thus, exclusion of thinner (early stage) lesions could lead to overestimation of *MC1R* associations. Further, in two studies, the case group consisted, in part, of individuals selected for increased likelihood of an underlying genetic susceptibility to melanoma (4, 7). This selection was severe in one study for which selected case subjects accounted for 73% of the case group (7). Specialized selection of case subjects may have led to overestimates of true associations between *MC1R* and melanoma status.

We must, however, consider the possibility that our study design contributed to the possible underestimation of the true *MC1R*-melanoma effect. Our study design is adapted from traditional case-control methodology, in which both cases and controls arise from a theoretical joint source population. Here, cases (individuals with multiple melanomas) arise from a population of at-risk persons with incident single primary melanoma (controls). The rationale for implementing this design was motivated by the desire to create an efficient population-based study for investigating genetic variants in *CDKN2A* and *CDK4* that are highly penetrant in the arena of familial melanoma (25) but rare at the population level (26). The degree to which this design may affect associations related to common exposures, including *MC1R* variants, is unknown. It is plausible that, among a population already at increased risk for melanoma (e.g., controls with single primary melanoma), the relative effect of exposures may be less than among a population at baseline risk (e.g., healthy controls with no history of melanoma), although the absolute effect on risk may be the same or greater (5).

Our stratified analysis by phenotypic index revealed that the overall pattern of association between melanoma and *MC1R* variants is not different across levels of cutaneous phenotype. Because of differences in the definition of "high-risk" *MC1R* variants, it was not possible to directly compare our results with those of Palmer et al. (4), Landi et al. (8), or Dwyer et al. (27), who found stronger associations between melanoma and *MC1R* variants among individuals with darker pigmentation.

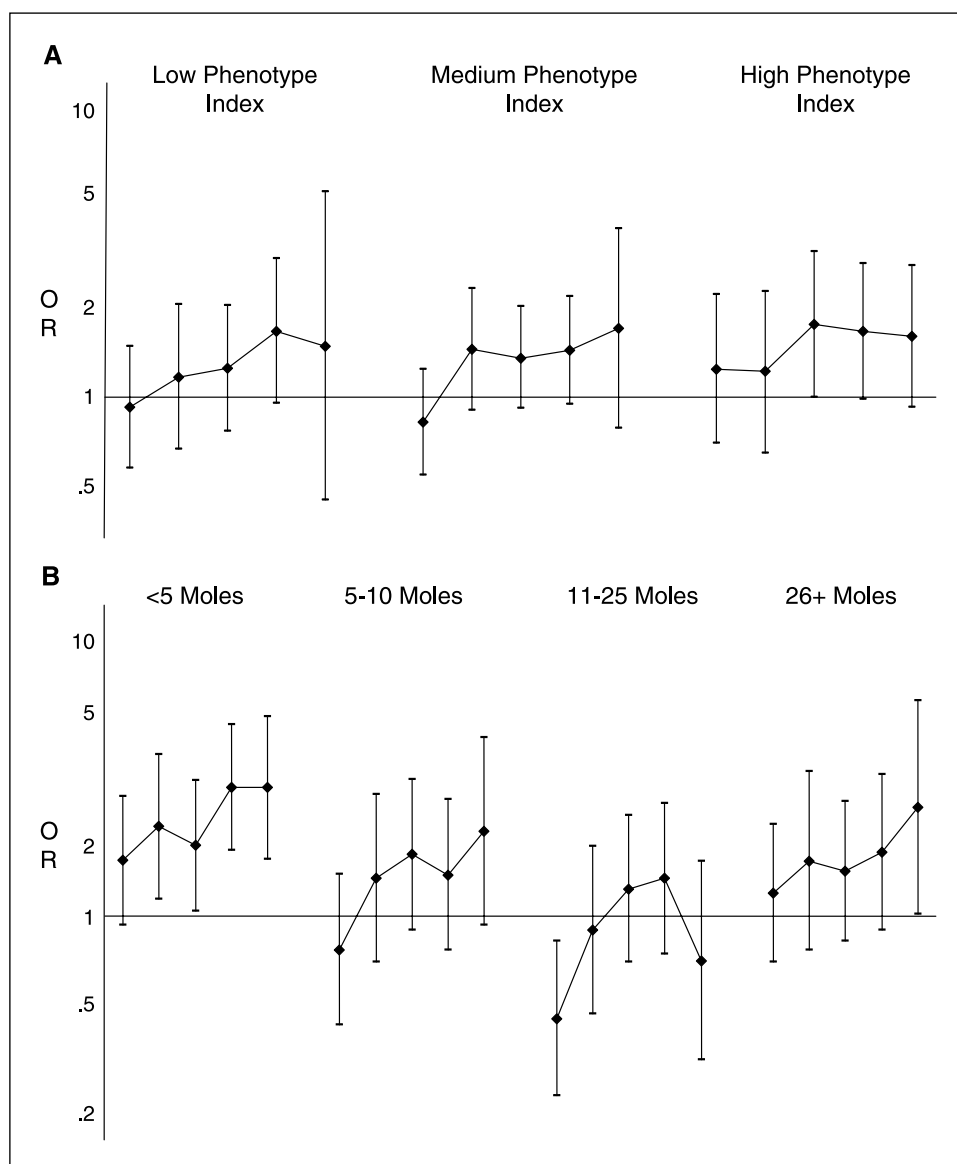


Figure 1. Association of *MC1R* variants and melanoma stratified by phenotype. Within each strata, aORs and 95% CIs are shown for five *MC1R* risk categories (left to right, con/r, r/r, con/R, R/r, and R/R). The reference genotype group for all associations is carriage of two copies of the *MC1R* consensus sequence (con/con). **A**, association of *MC1R* variants and melanoma stratified by phenotypic index. The phenotypic index reflects an individual's hair color, eye color, and inability to tan in response to sun exposure. A score of 1 or 2 (low) indicates overall darker cutaneous phenotype, whereas a score of 4 or 5 (high) indicates overall fairer cutaneous phenotype. **B**, association of *MC1R* variants and melanoma stratified by self mole count. Mole count categories reflect an individual's self-reported count of nevi on the back.

For a subset of individuals, DNA did not amplify. Individuals whose DNA amplified ($n = 3,218$) compared with those whose DNA did not amplify ($n = 366$) were slightly older at their most recent melanoma diagnosis (60 versus 58 years; $P = 0.05$), more likely to be male (64% versus 55%; $P < 0.001$), and more likely from Australia (59% versus 31%) than from North America (37% versus 67%) or Italy (4% versus 2%; $P < 0.001$, for comparison of the three geographic regions). There was not a statistically significant difference between the groups for report of moles on the back ($P = 0.06$, for comparison of the four mole categories) or distribution of the phenotypic index ($P = 0.18$, for comparison of the three categories of phenotypic index). Importantly, we found no difference in genotyping success by case status ($P = 0.12$). Therefore, it is unlikely that the addition of these 366 individuals to our analyses would have had a significant effect on our point estimates.

Because our control group consisted of individuals with incident first primary melanoma rather than those with prevalent first primary melanoma, we also assessed the effect of *MC1R* variants on survival by examining time from ultimate to penultimate

diagnosis of melanoma among multiple primary cases within individual variants and by carriage number (0 versus ≥ 1 variants). There were no statistically significant differences in the mean number of years between melanoma diagnoses, although difference for the R142H and D294H variants approached borderline statistical significance ($P = 0.07$ and 0.10, respectively).

This study of >3,200 individuals ascertained predominately from population-based sources and for whom complete genotype information at the *MC1R* locus was available is the largest molecular epidemiologic investigation of melanoma susceptibility to date. Although the point estimates of melanoma-*MC1R* associations were smaller than previously observed and our attenuated estimates may have affected our ability to definitively confirm differences in the association between *MC1R* variants and melanoma among individuals with different phenotypic profiles, it is noteworthy that our finding of a positive association between melanoma and *MC1R* variants and a trend toward increasingly stronger estimates with increasing *MC1R* risk category based on carriage number and variant (R or r) type is consistent with previous investigations.

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