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Melanocortin 1 receptor variants and skin cancer risk

Jiali Han[1,2]*, Peter Kraft[2,3], Graham A. Colditz[1,3], Jason Wong[1] and David J. Hunter[1–3]

[1]*Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*
[2]*Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA*
[3]*Department of Epidemiology, Harvard School of Public Health, Boston, MA*

Melanocortin 1 receptor (MC1R) gene variants are associated with red hair and fair skin color. We assessed the associations of common MC1R genotypes with the risks of 3 types of skin cancer simultaneously in a nested case–control study within the Nurses' Health Study (219 melanoma, 286 squamous cell carcinoma (SCC), and 300 basal cell carcinoma (BCC) cases, and 873 controls). We found that the 151Cys, 160Trp and 294His variants were significantly associated with red hair, fair skin color and childhood tanning tendency. The MC1R variants, especially the 151Cys variant, were associated with increased risks of the 3 types of skin cancer, after controlling for hair color, skin color and other skin cancer risk factors. Carriers of the 151Cys variant had an OR of 1.65 (95% CI, 1.04–2.59) for melanoma, 1.67 (1.12–2.49) for SCC and 1.56 (1.03–2.34) for BCC. Women with medium or olive skin color carrying 1 nonred hair color allele and 1 red hair color allele had the highest risk of melanoma. A similar interaction pattern was observed for red hair and carrying at least 1 red hair color allele on melanoma risk. We also observed that the 151Cys variant contributed additional melanoma risk among red-haired women. The information on MC1R status modestly improved the risk prediction; the increase was significant for melanoma and BCC (*p*, 0.004 and 0.05, respectively). These findings indicated that the effects of the MC1R variants on skin cancer risk were independent from self-reported phenotypic pigmentation.

Key words: MC1R; skin cancer; melanoma; skin color; hair color

Skin cancer is the most common form of cancer in the United States and accounts for approximately 1 million new cases per year, including about 55,000 cases of cutaneous malignant melanoma (hereafter called melanoma).[1,2] There are 3 major types of skin cancer. Melanoma is the most fatal form. The most common type of nonmelanoma skin cancer is basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC). The carcinogenic effects of ultraviolet (UV) radiation have been demonstrated in the etiology of both melanoma and nonmelanoma skin cancer.[3–5] Dark pigmentation is one important inherited protective factor for UV-induced skin cancer. Pigmentary melanin is synthesized in melanocytes and secreted into keratinocytes. Brown/black eumelanin absorbs UV and neutralizes free radicals to protect skin from UV damage, whereas yellow/red phaeomelanin generates free radicals in response to UV.

Human pigmentation is an inherited trait partially determined by the melanocortin 1 receptor (*MC1R*) gene. The *MC1R* gene is located at 16q24.3.[6] It encodes a 317-amino acid 7-pass-transmembrane G protein coupled receptor. Activated by α-melanocyte stimulating hormone (α-MSH) receptor or adrenocorticotrophic hormone (ACTH), MC1R activates adenylate cyclase enzyme, and thereby elevates intracellular cAMP. MC1R was shown to be the limiting factor controlling the output of the cAMP signaling pathway using heterologous cells expressing the wild-type *MC1R* gene.[7] This signaling induces the maturation of the phenomelanosome to the eumelanosome, resulting in darker pigmentation.[8,9]

A genotype–phenotype relationship has been reported for certain MC1R variants and light pigmentation. The human *MC1R* gene is highly polymorphic among light-pigmentation populations.[10,11] Among more than 80 nonsynonymous variants identified to date, the Arg151Cys, Arg160Trp and Asp294His were associated with red hair phenotype[12–14] and are known as "red hair color" (RHC) variants; other variants with weaker association were referred to as "nonred hair color" (NRHC) variants.

Several variants in the *MC1R* gene have been associated with increased risks for melanoma and nonmelanoma skin cancers after pigmentation phenotype was taken into account.[15–21] However, the joint effect of the MC1R variants and self-reported pigmentation on skin cancer risk is largely unknown. In this nested case–control study within the Nurses' Health Study, we evaluated the associations of 7 common MC1R variants (Val60Leu, Val92Met, Arg151Cys, Ile155Thr, Arg160Trp, Arg163Gln and Asp294His) with pigmentary phenotypes and the 3 major types of skin cancer (melanoma, SCC and BCC) simultaneously in US Caucasians. We examined the haplotypes inferred from the 7 common MC1R variants. In addressing the independent effect of the MC1R variants on skin cancer risk from self-reported phenotypic pigmentation, we assessed whether the carriage of MC1R variants contributed additional skin cancer risk after controlling for hair color, skin color, and other risk factors, and evaluated the interactions between the MC1R variants and phenotypic pigmentation on skin cancer risk. In addition, we explored the improvement of risk prediction for skin cancers contributed by MC1R genetic status.

Material and methods

Study population

The Nurses' Health Study was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55 completed a self-administered questionnaire on their medical histories and baseline health-related exposures. Updated information has been obtained by questionnaires every 2 years. Between 1989 and 1990, blood samples were collected from 32,826 of the cohort members. The distributions of risk factors for skin cancer were very similar in the subcohort of those who donated blood samples as in the overall cohort. For example, we observed the following prevalence (blood subcohort vs. overall cohort): red or blonde hair (16 *vs.* 15%), childhood or adolescent tendency to burn (36 *vs.* 35%), childhood or adolescent tendency to deep tan (23 *vs.* 24%), and family history of melanoma (5 *vs.* 5%). Detailed methods of this nested case–control study were described previously.[22] Briefly, eligible cases in this study consisted of women with incident skin cancer from the subcohort who had given a blood specimen, including SCC and BCC cases with a diagnosis anytime after blood collection up to June 1, 1998 and melanoma cases up to June 1, 2000 with no previously diagnosed skin cancer. A common control series was randomly selected from participants who gave a blood sample and were free of diagnosed skin cancer up to and including the questionnaire cycle in which the case was diagnosed. One or two controls

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; NRHC, nonred hair color allele; OR, odds ratio; RHC, red hair color allele; ROC, receiver-operating characteristic; SCC, squamous cell carcinoma; UV, ultraviolet; WT, consensus allele.

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***Correspondence to:** Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Ave, Boston, MA 02115, USA. Fax: +617-525-2008. E-mail: jiali.han@channing.harvard.edu

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were matched to each case by year of birth (±1 year) and self-reported race (Caucasian/missing or others). Fewer than 5% of cases and controls had missing or other race/ethnicity. The nested case–control study consisted of 219 melanoma cases (including 77 *in situ* cases), 286 SCC cases, 300 BCC cases and 873 matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

Exposure data

Information regarding skin cancer risk factors was obtained from the prospective biennial questionnaires and the retrospective supplementary questionnaire. Information on natural hair color and childhood and adolescent tendency to sunburn or tan was asked in the 1982 prospective questionnaire; ethnic group in the 1992 questionnaire. The retrospective supplementary questionnaire consisted of questions in 3 major areas: (*i*) pigmentation, constitutional, and susceptibility factors; (*ii*) history of residence (states and towns), sun exposure habits, and severe sunburns at different ages; and (*iii*) family history of skin cancer (father, mother and siblings). We mailed to 758 living cases and 804 living controls the supplementary questionnaire. The participation rate was 92% for the cases and 89% for the controls. In addition, the 11 states of residence of cohort members at baseline were grouped into 3 regions: Northeast (Connecticut, Massachusetts, Maryland, New Jersey, New York and Pennsylvania), Northcentral (Michigan and Ohio) and West and South (California, Texas and Florida). Estimation of past sunlight exposure for each subject was described previously.[22]

Single nucleotide polymorphism identification

The distribution and frequency of MC1R variants in 179 Caucasian controls from the US were determined by Kanetsky *et al*. using a direct sequencing method.[23] Seven nonsynonymous polymorphisms with allele frequency above 1% were identified (Val60Leu, Val92Met, Arg151Cys, Ile155Thr, Arg160Trp, Arg163Gln and Asp294His) in the coding region. We genotyped them in our case–control study. We did not genotype the 3 variants (86insA, Asp84Glu and Arg142His) with allele frequency of 1% or less.

Laboratory assays

The Arg160Trp polymorphism was genotyped by restriction fragment length polymorphism (RFLP), and the other polymorphisms were genotyped by the 5′ nuclease assay (TaqMan®) in 384-well format, using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case–control status and blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request.

Statistical methods

We used a common control series in data analyses to increase the statistical power. In addition to analyzing each variant individually, we also classified the variants as follows; the Arg151Cys, Arg160Trp and Asp294His variants were categorized as "red hair color" (RHC) variants, other 4 variants were referred to as "nonred hair color" (NRHC) variants.[12–14] We defined the chromosome without the variant at each of the 7 polymorphic sites that we genotyped as the consensus allele (WT). We used a χ^2 test to assess whether the MC1R genotypes were in Hardy-Weinberg equilibrium among the controls and the relation of the genotypes with natural hair color, skin color and childhood tendency to tan among the controls. Based on the 7 variants, the EM algorithm was run to estimate haplotype frequencies in cases and controls.[24,25] Without resequencing the gene, the haplotype analysis was to examine the phase of these multiple nonsynonymous polymorphisms genotyped in this study. Unconditional logistic regression was employed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to assess the risk of skin cancer for each genotype. In the analyses of each polymorphism, the main effect was evaluated using the wildtype of the consensus sequence as the reference group. The OR was calculated for the risk for 1 additional variant allele of each polymorphism as used previously.[16,18]

Interactions between the MC1R genotypes and skin color or hair color on skin cancer risks were evaluated in unconditional logistic regression models. We used cross-classified categories of the genotype and hair color or skin color compared to a common reference category. In the interaction analyses between the MC1R variants and skin color on skin cancer risk, we evaluated the following categories; 2 groups of skin color: fair versus medium or olive; 4 groups of genotypes: WT/WT, WT/NRHC or NRHC/NRHC, WT/RHC or RHC/RHC, versus NRHC/RHC. The common reference group was women with fair skin color and 2 consensus alleles (WT/WT). In the interaction analyses between the MC1R variants and hair color on skin cancer risk, we evaluated the following categories. Four groups of hair color: black or dark brown, light brown, blonde, versus red; 3 groups of genotype: WT/WT, WT/NRHC or NRHC/NRHC, versus WT/RHC or RHC/RHC or NRHC/RHC (WT/RHC, RHC/RHC and NRHC/RHC were grouped together due to sparse cells). The common reference group was women who had black or dark brown hair and carried 2 consensus alleles (WT/WT). In the interaction analyses between the individual MC1R variant and hair color, we modeled hair color as an ordinal variable and the MC1R genotype as a dichotomous variable (carriers *vs*. noncarriers) to test the statistical significance of interaction. The test of a single multiplicative interaction term assessed whether the trend for hair color was significantly different according to the genotype.

We examined whether the MC1R status added to risk prediction for skin cancers based on phenotypic variables. We estimated the concordance statistic, which represents the probability that for a randomly selected pair of individuals, one diseased and one nondiseased, the diseased individual has the higher estimated disease probability. Thus, this is an index of predictive discrimination based on the rank correlation between predicted and observed outcomes. The concordance statistic can range from 0.5 to 1.0. The concordance statistic is equivalent to the area under a receiver-operating characteristic (ROC) curve. All statistical tests were two-sided.

Results

Descriptive characteristics of cases and controls

At the beginning of the follow-up of this nested case–control study at blood collection, the women were between 43 and 68 years old with the mean age of 58.7 years. The mean age at diagnosis of incident melanoma cases was 63.4 years and that of SCC cases and BCC cases was 64.7 and 64.0 years, respectively. Basic characteristics of cases and controls in this study are presented in Table I. Women in the West and South regions were more likely to be diagnosed with SCC or BCC compared to those in the Northeast. Skin cancer cases, especially melanoma cases, were more likely to possess red hair color and fair skin color. Cases of each type of skin cancer were more likely to have used sunlamps or attended tanning salons. A family history of skin cancer was a risk factor for the 3 types of skin cancer. Cases of each type of skin cancer were more likely to have had higher cumulative sun exposure while wearing a bathing suit, and have more lifetime severe sunburns that blistered (Table I).

Frequency distribution of MC1R variants

The genotype distributions of the 7 nonsynonymous polymorphisms were in Hardy-Weinberg equilibrium among controls. The allele frequency of the 7 polymorphisms among the controls was 12.7% for Val60Leu, 9.8% for Val92Met, 7.0% for Arg151Cys, 1.3% for Ile155Thr, 7.8% for Arg160Trp, 4.0% for Arg163Gln

TABLE I – CHARACTERISTICS OF SKIN CANCER CASES AND CONTROLS IN THE NESTED CASE–CONTROL STUDY

Characteristic	Controls (n = 873)	Melanoma cases (n = 219)	SCC cases (n = 286)	BCC cases (n = 300)
Age at diagnosis (mean, years)	64.5	63.4	64.7	64.0
Geographic region at baseline (%)				
Northeast	55.2	58.0	51.7	49.3
Northcentral	23.4	16.9	17.1	20.3
West and South	21.4	25.1	31.1	30.3
Natural hair color at age 20 (%)				
Black or dark brown	43.9	31.5	41.3	30.3
Light brown	40.0	42.5	34.6	45.7
Blonde	12.0	15.5	16.8	18.0
Red	2.9	10.5	5.2	4.7
Natural skin color (%)				
Fair	40.0	57.1	54.6	53.0
Medium	36.7	25.6	32.2	31.3
Olive	4.8	0.9	1.8	1.3
Sunlamp use or tanning salon attendance (%)	10.0	19.2	14.3	14.7
Family history of skin cancer (%)	25.1	36.5	35.7	42.7
Highest tertile of cumulative sun exposure with a bathing suit (%)	33.4	53.3	46.1	42.6
Number of lifetime severe sunburns (mean)	5.4	9.6	7.8	8.2

The percentages may not sum to 100 due to rounding.

TABLE II – THE ASSOCIATIONS OF *MC1R* GENOTYPES WITH PHENOTYPES AMONG CONTROLS

	WT/WT	WT/NRHC	NRHC/NRHC	WT/RHC	RHC/RHC	NRHC/RHC
Natural hair color at age 20						
Black and Dark Brown	118 (34.0)	124 (35.7)	36 (10.4)	50 (14.4)	0 (0)	19 (5.5)
Light Brown	96 (29.7)	105 (32.5)	20 (6.2)	65 (20.1)	4 (1.2)	33 (10.2)
Blonde	27 (27.8)	19 (19.6)	12 (12.4)	21 (21.7)	6 (6.2)	12 (12.4)
Red	0 (0.0)	0 (0.0)	0 (0.0)	8 (36.4)	14 (63.6)	0 (0.0)
Chi-Square Test		0.40	0.18	<0.001	<0.001	0.02
Natural skin color						
Fair	80 (25.1)	91 (28.5)	25 (7.8)	58 (18.2)	23 (7.2)	42 (13.2)
Medium	105 (35.4)	96 (32.3)	25 (8.4)	59 (19.9)	1 (0.3)	11 (3.7)
Olive	22 (57.9)	11 (29.0)	3 (7.9)	0 (0.0)	0 (0.0)	2 (5.3)
Chi-Square Test		0.10	0.38	<0.001	<0.001	<0.001
Childhood tendency to tan						
Practically none	13 (13.0)	17 (17.0)	16 (16.0)	23 (23.0)	12 (12.0)	19 (19.0)
Light tan	45 (22.8)	62 (31.5)	18 (9.1)	40 (20.3)	9 (4.6)	23 (11.7)
Average tan	126 (34.1)	127 (34.3)	29 (7.8)	66 (17.8)	3 (0.8)	19 (5.1)
Tan	56 (46.3)	42 (34.7)	5 (4.1)	15 (12.4)	0 (0.0)	3 (2.5)
Chi-Square Test		0.16	<0.001	<0.001	<0.001	<0.001

The percentages may not sum to 100 due to rounding. WT, consensus allele; NRHC, nonred hair color allele; RHC, red hair color allele. χ^2 tests were performed to compare each of the categories to the WT/WT group.

and 1.6% for Asp294His, which were compatible with the previous report on 179 US Caucasian controls.[23] Overall, based upon these 7 polymorphisms genotyped, 31% of the controls were homozygous for consensus allele. Half of the controls carried 1 variant allele; 32% carried 1 NRHC allele and 18% carried 1 RHC allele. 20% of the controls carried 2 variant alleles; 9% carried 2 NRHC alleles, 8% carried 1 NRHC allele and 1 RHC allele, and 3% carried 2 RHC alleles.

Associations of MC1R variants with hair color, skin color and childhood tanning tendency among the controls

We examined the associations between the MC1R variants and natural hair color at age 20, skin color, and childhood tanning tendency among the controls. In the analyses of each variant, women carrying one of the RHC alleles were more likely to have red hair color, fair skin color or less childhood tendency to tan (all p for χ^2 test < 0.02). Women with the 92Met allele were less likely to have red hair color (p for χ^2 test, 0.01); none of the red-haired women carried this allele. There was no association between this variant and skin color. There were 2 homozygotes for the 151Cys variant allele, one with red hair color and the other with blonde hair color. We observed 8 homozygotes for the 160Trp variant allele, 4 with red hair color, 2 with blonde hair color, and 2 with light brown hair color. These 10 homozygotes all had fair skin color, and all reported light tanning or no tanning tendency in childhood. There was no homozygote for the 294His variant allele.

We also examined the combined effect of these variants (Table II). We divided participants into 6 categories according to carriage of consensus allele, NRHC and RHC variants. Among the 22 red-haired controls, 8 had 1 consensus allele and 1 RHC allele, and 14 had 2 RHC alleles. Compared to women with 2 consensus alleles, those carrying at least 1 RHC allele were more likely to have lighter hair color and fairer skin color. The carriage of at least 1 RHC allele or 2 NRHC alleles was significantly associated with less childhood tendency to tan.

Haplotype analysis on the 7 common MC1R variants

The haplotype analysis was to examine the phase of the multiple polymorphisms genotyped in this study. There were 8 common haplotypes inferred from the 7 polymorphisms, which accounted for 99% of the chromosomes of the study populations (Table III). These 7 variants were mutually exclusive from each other on these common haplotypes. One haplotype was the consensus allele; each of the other 7 haplotypes carried only 1 variant allele of the 7 sites exclusively. Therefore, the carriage of a polymorphism can be viewed as the carriage of the corresponding haplotype in the evaluation of the main effect of polymorphic sites and gene-environment interactions.

TABLE III – *MC1R* HAPLOTYPES AND SKIN CANCER RISK

Val60Leu	Val92Met	Arg151Cys	Ile155Thr	Arg160Trp	Arg163Gln	Asp294His	Controls *n* (%)	Melanoma Cases *n* (%)	SCC Cases *n* (%)	BCC Cases *n* (%)
0	0	0	0	0	0	0	973 (55.8)	186 (42.4)	265 (46.3)	287 (48.0)
1	0	0	0	0	0	0	223 (12.8)	58 (13.1)	79 (13.9)	85 (14.3)
0	1	0	0	0	0	0	170 (9.7)	48 (10.9)	56 (9.8)	57 (9.4)
0	0	0	0	1	0	0	137 (7.8)	42 (9.6)	63 (11.0)	50 (8.4)
0	0	1	0	0	0	0	122 (7.0)	57 (13.0)	64 (11.2)	67 (11.3)
0	0	0	0	0	1	0	69 (3.9)	22 (5.1)	21 (3.7)	35 (5.8)
0	0	0	0	0	0	1	27 (1.6)	16 (3.7)	13 (2.3)	12 (2.1)
0	0	0	1	0	0	0	23 (1.3)	8 (1.9)	9 (1.6)	4 (0.7)

"0" stands for wildtype allele; "1" stands for variant allele.

Associations of MC1R variants with skin cancer risk

The main effect of each polymorphism was evaluated using the wildtype of the consensus sequence as the reference group (Table IV). For the 3 RHC variants, in the analyses only controlling for matching factors, the 151Cys carriers had a significantly increased risk for each type of skin cancer; the carriage of the 160Trp allele was significantly associated with melanoma and SCC risks; the 294His carriers had a significantly increased risk of melanoma. After additionally controlling for hair color, skin color and other risk factors, the risks were attenuated. Only the carriage of the 151Cys allele remained significant for melanoma, SCC and BCC.

For the NRHC alleles, the 92Met allele was inversely associated with light hair color, and was significantly associated with an increased melanoma risk. The 163Gln allele was not correlated with hair color or skin color, but was associated with increased risk of BCC. These associations remained significant after controlling for all risk factors in multivariate models.

There was no substantial difference in the main effect of each variant on the 3 types of skin cancer. For melanoma, the analyses excluding the *in situ* cases yielded similar results, although our power to detect heterogeneity was limited.

Associations of combined MC1R variants with skin cancer risk

The combined effect of MC1R variants on skin cancer risk was shown in Table V. Overall, the skin cancer risks associated with NRHC alleles were not attenuated substantially after controlling for other risk factors. Women with 1 consensus allele and 1 NRHC allele had a significantly increased melanoma risk after controlling for pigmentation and other risk factors. Women with 2 NRHC alleles had a significantly increased risk of BCC in the multivariate models. For RHC variants, after controlling for pigmentation and other risk factors, the significant associations for the 3 types of skin cancer were attenuated and became nonsignificant among women with 1 consensus allele and 1 RHC allele as well as those with 2 RHC alleles. The strongest association was found among women with 1 RHC allele and 1 NRHC allele for melanoma. After additionally controlling for skin color, hair color and other risk factors, the risk was attenuated from 4-fold to 2.6-fold and remained significant. A similar effect was found for SCC among this group of women.

Interactions between MC1R variants and skin color and hair color on skin cancer risk

We evaluated the interaction between the MC1R variants and skin color on skin cancer risk (Fig. 1). Compared to women with fair skin color and 2 consensus alleles (WT/WT), we found that the effect of carrying 1 NRHC allele and 1 RHC allele (NRHC/RHC) was strongest for melanoma risk among women with medium or olive skin color (OR, 4.66; 95% CI, 1.50–14.49); all other cross-classified categories of skin color and genotype had ORs less than 2.2 (p, interaction, 0.06). Unlike melanoma, no such interaction was found for the risk of SCC or BCC (Fig. 1).

We also evaluated the interaction between the MC1R variants and hair color on skin cancer risk. All red-haired women carried at least 1 RHC allele; hence no red-haired women were in the groups of WT/WT and WT/NRHC or NRHC/NRHC. In the cross-classified interaction analyses for melanoma risk, compared to women who had black or dark brown hair and carried 2 consensus alleles (WT/WT), red-haired women carrying at least 1 RHC allele (WT/RHC, RHC/NRHC and RHC/RHC) had an OR of 5.61 (95% CI, 2.31–13.66) after controlling for skin color and other risk factors; all other cross-classified categories had nonsignificant ORs less than 2.0 (p, interaction, 0.03). There was no such joint effect of red hair and the carriage of at least 1 RHC allele for either SCC or BCC.

When we examined the variants individually, we observed a joint effect of the 151Cys allele and red hair color on melanoma risk (Fig. 2). The carriage of the 151Cys allele was associated with an increased risk of melanoma among the women with red hair color, but not among those with other hair color. Compared to women with black/dark brown hair color and Arg/Arg genotype (59 cases and 332 controls), the 151Cys carriers with red hair color (17 cases and 11 controls) had a significantly increased risk of melanoma (OR, 7.24; 95% CI, 2.96–17.67), whereas the red-haired women with Arg/Arg genotype (6 cases and 13 controls) had an OR of 2.19 (95% CI, 0.69–6.90) (p for interaction, 0.03). In the stratified analyses by hair color, among women with red hair color the risk of melanoma for 1 additional 151Cys allele was 3.28 (95% CI, 1.32–8.14). There was no significant association between the 151Cys allele and the risk in other strata of hair color. No such interaction was observed for SCC or BCC risk.

No interaction was observed between any other individual variants and hair color on skin cancer risk. We did not observe any interactions between these variants individually and skin color on skin cancer risk.

Risk prediction contributed by the MC1R genetic status

We performed an exploratory analysis to evaluate how much additional predictive ability the genetic variants in the MC1R gene added to the risk prediction model only containing nongenetic host factors, such as age, hair color, skin color, the number of moles, childhood and adolescence tendency to burn, the number of lifetime severe sunburn and family history of skin cancer. We added 1 variable for each allele and an interaction term for the 151Cys allele and hair color to the above model. The concordance statistic is presented in Table VI. The increase in the concordance statistic contributed by the MC1R genetic variants ranged from 0.012 to 0.020, which suggested that the information on MC1R status had modest ability to improve the risk prediction; the increase was significant for melanoma and BCC.

Discussion

We evaluated the associations of the MC1R variants with pigmentary phenotypes and the risks of 3 types of skin cancers simultaneously among US Caucasians. After controlling for self-reported pigmentary phenotypes and other skin cancer risk factors, the multivariate analyses showed independent effects of specific MC1R variants on skin cancer risk. Women with medium or olive skin color carrying 1 NRHC allele and 1 RHC allele had the highest risk of melanoma. A similar interaction pattern was observed

TABLE IV – *MC1R* GENOTYPES AND SKIN CANCER RISK[1]

	Melanoma			SCC			BCC		
	Val/Val	Val/Leu	Leu/Leu	Val/Val	Val/Leu	Leu/Leu	Val/Val	Val/Leu	Leu/Leu
Val60Leu									
Cases/controls	159/659	55/183	1/18	211/659	69/183	5/18	217/659	70/183	7/18
Multivariate OR[2]	1.00	1.38 (0.97–1.96)		1.00	1.36 (1.01–1.85)		1.00	1.36 (1.01–1.83)	
Multivariate OR[3]	1.00	1.28 (0.90–1.83)		1.00	1.30 (0.96–1.77)		1.00	1.31 (0.97–1.78)	
Multivariate OR[4]	1.00	1.21 (0.83–1.76)		1.00	1.27 (0.92–1.75)		1.00	1.24 (0.90–1.71)	
Val92Met	Val/Val	Val/Met	Met/Met	Val/Val	Val/Met	Met/Met	Val/Val	Val/Met	Met/Met
Cases/controls	165/707	44/149	2/10	230/707	54/149	1/10	243/707	48/149	4/10
Multivariate OR[2]	1.00	1.56 (1.08–2.27)		1.00	1.19 (0.84–1.69)		1.00	1.04 (0.73–1.49)	
Multivariate OR[3]	1.00	1.57 (1.07–2.29)		1.00	1.15 (0.81–1.64)		1.00	1.04 (0.72–1.48)	
Multivariate OR[4]	1.00	1.56 (1.05–2.32)		1.00	1.09 (0.75–1.59)		1.00	0.96 (0.65–1.40)	
Arg151Cys	Arg/Arg	Arg/Cys	Cys/Cys	Arg/Arg	Arg/Cys	Cys/Cys	Arg/Arg	Arg/Cys	Cys/Cys
Cases/controls	162/725	40/113	8/2	220/725	58/113	3/2	227/725	60/113	3/2
Multivariate OR[2]	1.00	2.49 (1.70–3.64)		1.00	2.10 (1.47–2.99)		1.00	2.02 (1.41–2.88)	
Multivariate OR[3]	1.00	1.75 (1.14–2.69)		1.00	1.84 (1.27–2.67)		1.00	1.71 (1.17–2.51)	
Multivariate OR[4]	1.00	1.65 (1.04–2.59)		1.00	1.67 (1.12–2.49)		1.00	1.56 (1.03–2.34)	
Ile155Thr	Ile/Ile	Ile/Thr	Thr/Thr	Ile/Ile	Ile/Thr	Thr/Thr	Ile/Ile	Ile/Thr	Thr/Thr
Cases/controls	203/842	7/23	1/0	274/842	9/23	0/0	295/842	4/23	0/0
Multivariate OR[2]	1.00	1.99 (0.90–4.42)		1.00	1.61 (0.72–3.60)		1.00	0.51 (0.15–1.74)	
Multivariate OR[3]	1.00	1.82 (0.81–4.07)		1.00	1.43 (0.63–3.22)		1.00	0.44 (0.13–1.53)	
Multivariate OR[4]	1.00	1.47 (0.60–3.58)		1.00	1.28 (0.53–3.06)		1.00	0.32 (0.09–1.22)	
Arg160Trp	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Arg	Arg/Trp	Trp/Trp
Cases/controls	175/735	42/119	0/8	224/735	54/119	4/8	246/735	50/119	0/8
Multivariate OR[2]	1.00	1.59 (1.05–2.41)		1.00	1.83 (1.30–2.58)		1.00	1.44 (0.99–2.08)	
Multivariate OR[3]	1.00	1.17 (0.75–1.85)		1.00	1.61 (1.10–2.34)		1.00	1.22 (0.82–1.82)	
Multivariate OR[4]	1.00	1.08 (0.67–1.74)		1.00	1.45 (0.97–2.17)		1.00	1.11 (0.73–1.68)	
Arg163Gln	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Arg	Arg/Gln	Gln/Gln
Cases/controls	191/793	20/66	1/1	260/793	22/66	0/1	257/793	34/66	0/1
Multivariate OR[2]	1.00	1.69 (0.98–2.93)		1.00	1.21 (0.72–2.05)		1.00	1.99 (1.26–3.15)	
Multivariate OR[3]	1.00	1.67 (0.96–2.91)		1.00	1.24 (0.73–2.11)		1.00	1.93 (1.21–3.07)	
Multivariate OR[4]	1.00	1.63 (0.90–2.95)		1.00	1.27 (0.72–2.23)		1.00	2.08 (1.27–3.43)	
Asp294His	Asp/Asp	Asp/His	His/His	Asp/Asp	Asp/His	His/His	Asp/Asp	Asp/His	His/His
Cases/controls	200/830	16/27	0/0	267/830	14/27	0/0	276/830	12/27	0/0
Multivariate OR[2]	1.00	2.66 (1.34–5.28)		1.00	1.55 (0.75–3.22)		1.00	1.47 (0.71–3.06)	
Multivariate OR[3]	1.00	1.70 (0.81–3.57)		1.00	1.33 (0.62–2.86)		1.00	1.19 (0.56–2.52)	
Multivariate OR[4]	1.00	1.55 (0.71–3.41)		1.00	1.28 (0.57–2.85)		1.00	1.05 (0.48–2.31)	

[1]The number of participants does not sum to total women because of missing data on genotype. The OR was calculated for the risk for one additional variant allele of each polymorphism.–[2]Unconditional logistic regression adjusted for the matching variables: age and race (Caucasian/missing).–[3]Unconditional logistic regression adjusted for the matching variables, natural skin color, natural hair color.–[4]Unconditional logistic regression adjusted for the variables in model C, along with childhood tendency to burn, palpably moles on arms, family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.

TABLE V – COMBINED EFFECT OF MC1R AND SKIN CANCER RISK[1]

	Controls *n* (%)	Melanoma	SCC	BCC
		Cases *n* (%)	Cases *n* (%)	Cases *n* (%)
WT/WT	244 (30.5)	32 (16.1)	52 (19.6)	56 (21.0)
Multivariate OR[2]		1.00	1.00	1.00
Multivariate OR[3]		1.00	1.00	1.00
Multivariate OR[4]		1.00	1.00	1.00
WT/NRHC	252 (31.5)	61 (30.7)	77 (29.1)	71 (26.6)
Multivariate OR[2]		1.84 (1.16–2.93)	1.44 (0.97–2.13)	1.22 (0.82–1.80)
Multivariate OR[3]		1.75 (1.09–2.79)	1.41 (0.95–2.10)	1.19 (0.80–1.78)
Multivariate OR[4]		1.64 (1.00–2.67)	1.32 (0.87–2.00)	1.11 (0.73–1.68)
NRHC/NRHC	70 (8.8)	16 (8.0)	20 (7.5)	34 (12.7)
Multivariate OR[2]		1.71 (0.89–3.31)	1.35 (0.75–2.41)	2.14 (1.30–3.55)
Multivariate OR[3]		1.63 (0.84–3.18)	1.25 (0.70–2.26)	2.03 (1.22–3.39)
Multivariate OR[4]		1.63 (0.80–3.30)	1.16 (0.63–2.14)	1.80 (1.05–3.10)
WT/RHC	145 (18.1)	43 (21.6)	61 (23.0)	65 (24.3)
Multivariate OR[2]		2.20 (1.33–3.65)	1.99 (1.30–3.04)	1.96 (1.29–2.96)
Multivariate OR[3]		1.77 (1.05–2.97)	1.83 (1.18–2.83)	1.72 (1.13–2.62)
Multivariate OR[4]		1.71 (0.99–2.95)	1.56 (0.99–2.46)	1.48 (0.95–2.31)
RHC/RHC	25 (3.1)	14 (7.0)	14 (5.3)	12 (4.5)
Multivariate OR[2]		4.21 (1.98–8.97)	2.68 (1.30–5.50)	2.13 (1.00–4.51)
Multivariate OR[3]		1.29 (0.48–3.51)	1.72 (0.74–4.01)	1.27 (0.51–3.17)
Multivariate OR[4]		1.09 (0.36–3.26)	1.53 (0.64–3.68)	1.09 (0.42–2.81)
NRHC/RHC	64 (8.0)	33 (16.6)	41 (15.5)	29 (10.9)
Multivariate OR[2]		4.02 (2.29–7.05)	3.03 (1.85–4.96)	1.98 (1.17–3.35)
Multivariate OR[3]		2.98 (1.66–5.33)	2.59 (1.56–4.32)	1.61 (0.93–2.76)
Multivariate OR[4]		2.64 (1.43–4.90)	2.41 (1.41–4.10)	1.43 (0.81–2.52)

[1]WT stands for consensus allele. NRHC stands for non-red hair color allele. RHC stands for red hair color allele.–[2]Unconditional logistic regression adjusted for the matching variables: age and race (Caucasian/missing).–[3]Unconditional logistic regression adjusted for the matching variables, natural skin color, natural hair color.–[4]Unconditional logistic regression adjusted for the variables in model C, along with childhood tendency to burn, palpably moles on arms, family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.

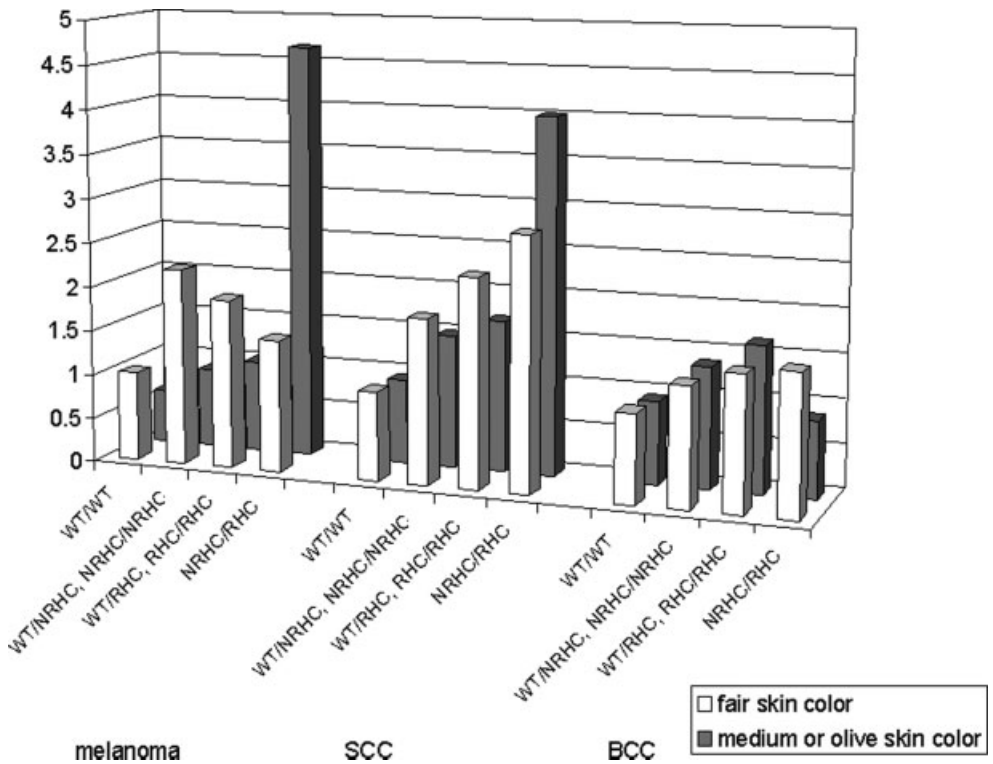

FIGURE 1 – Unconditional logistic regression adjusted for matching factors, hair color, skin color, childhood tendency to burn, palpably moles on arms, family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region. WT stands for consensus allele. NRHC stands for nonred hair color allele. RHC stands for red hair color allele. *p* for interaction, 0.06 for melanoma, 0.75 for SCC and 0.91 for BCC.

for red hair and carrying at least 1 RHC allele on melanoma risk. We also observed that the 151Cys variant contributed additional melanoma risk among red-haired women. MC1R status had reasonable ability to improve a risk prediction model with known phenotypic factors, especially for melanoma and BCC.

Given the highly polymorphic nature of this locus with over 80 variants identified, only genotyping common variants may result in misclassifying the allele without the observed common variants as the consensus sequence. Based on direct sequencing of MC1R on 179 US Caucasian controls,[23] counting only nonsynonymous and

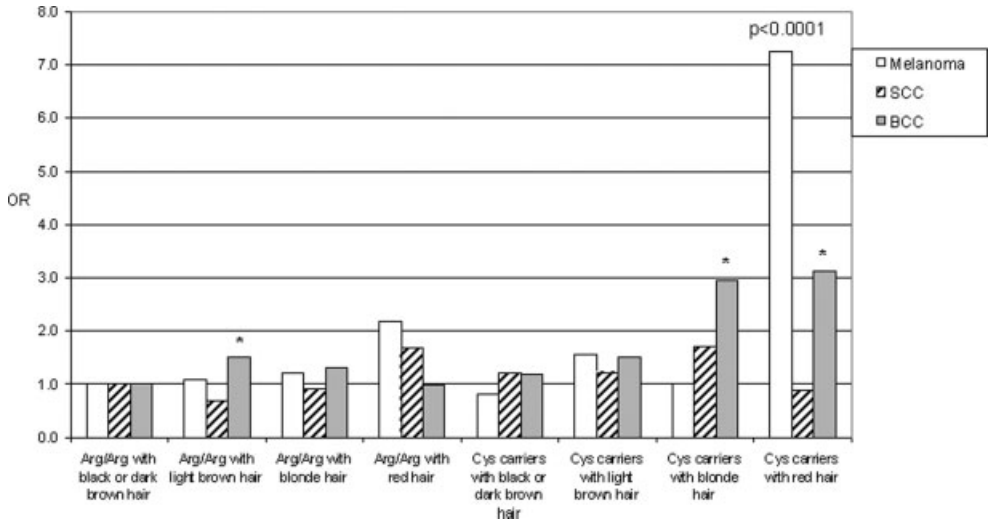

FIGURE 2 – Interaction with Arg151Cys and hair color on skin cancer risk, adjusted for age, race, natural skin color, childhood tendency to burn, palpably moles on arms, family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles) and geographic region. The reference group is the women with black/dark brown hair color and Arg/Arg genotype. The asterisk indicates that the risk for the specific category is significantly elevated from the reference group. *p* for interaction for melanoma risk is 0.03. No such interaction was observed for SCC or BCC risk.

TABLE VI – ADDITION OF MC1R GENETIC VARIANTS TO RISK PREDICTION FOR SKIN CANCERS

	Without genetic factors	With genetic factors	Increase value	*p*, *df* = 8
Melanoma	0.708	0.728	0.020	0.004
SCC	0.655	0.667	0.012	0.23
BCC	0.679	0.693	0.014	0.05

We added one variable for each allele and an interaction term for the 151Cys allele and hair color to the risk prediction model only containing non-genetic host factors, such as age, hair color, skin color, the number of moles, childhood and adolescence tendency to burn, the number of lifetime severe sunburn and family history of skin cancer.

one rare frameshift variant, 72% carried at least 1 variant, and 22% carried two. Overall, approximately 53% of the chromosomes had no such variants. In our haplotype analysis, we estimated that about 56% of the chromosomes among controls had none of the 7 common variants that we genotyped. In other words, by genotyping the 7 common variants, we likely only misclassified about 3% of the chromosomes as consensus alleles that may actually contain other rare nonsynonymous variants that we did not genotype.

The presence of specific MC1R variants was previously associated with quantitatively measured hair color,[26] and experimentally induced erythemal response to UV.[27] Among the known variants, the 3 RHC variants, 151Cys, 160Trp and 294His, have been reported most extensively to have functional impact. The RHC variants displayed decreased capacity of elevating cAMP levels after activation in *in vitro* transfection assays in COS-1 cells.[28,29] Scott *et al*. showed that cultured human melanocytes with the RHC variants had decreased capacity to stimulate cAMP upon activation and increased UV sensitivity.[30] The RHC variants had reduced function to rescue the *MC1R* deficiency in transgenic mice.[31] Homozygotes for 151Cys and 160Trp have the least melanin in cultured human melanocytes.[32] Recently, Newton *et al*. reported that the 3 RHC variants were not nonfunctional, with the Arg151Cys and Arg160Trp retaining considerable signaling capacity in stably transfected HEK293 cells and in melanoma cells.[33]

Tanning response is an increase in melanin production after UV exposure, which induces a-MSH and the expression of MC1R in the skin,[34,35] which triggers tyrosinase gene transcription and enzymatic activity.[36,37] We observed that the carriers of at least 1 RHC allele or 2 NRHC alleles were less likely to have a childhood tendency to tan after repeated sun exposure, which further supported that these variants were associated with a defect in activating cAMP signaling of melanin production.

Among the 7 polymorphisms examined, the 151Cys, 160Trp and 294His variants were individually or jointly associated with red hair color and fair skin color, which was consistent with previous studies reporting them as RHC alleles. However, no strict recessive mode of inheritance was observed. These associations helped to explain the attenuation of skin cancer risk associated with the 3 variants in multivariate models. Notably, however, even after controlling for self-reported pigmentation and other risk factors, the 151Cys allele remained associated with increased risks of the 3 types of skin cancer. This suggests that the 151Cys allele may independently confer skin cancer risk beyond its effect on self-reported pigmentary phenotype. For the NRHC alleles, the 92Met allele, which was inversely associated with light hair color, was significantly associated with an increased melanoma risk. The 163Gln allele was associated with increased risk of BCC. These significant associations were not attenuated after controlling for all risk factors in multivariate models, which suggests that the risks associated with these NRHC alleles may not solely mediated by pigmentary phenotypes.

The analyses on combined effects of RHC and NRHC alleles indicated that both RHC and NRHC alleles were associated with skin cancer risk. There was 4-fold-increased risk for melanoma among women who carried 1 NRHC allele and 1 RHC allele and those who carried 2 RHC alleles. After controlling for hair color, skin color and other risk factors, the risk among those with 2 RHC alleles was substantially attenuated, whereas the risk among those with 1 NRHC allele and 1 RHC allele remained significant and was about two-and-half fold. The risk of this group was the highest among the different combinations of wildtype, NRHC, and RHC alleles. This suggests that the NRHC and RHC alleles may have different and synergistic effect on melanoma risk. However, because of the small numbers in the group of women with 2 RHC alleles, this finding requires confirmation. A similar effect was observed for SCC risk.

Palmer *et al*.[16] observed that the association of the MC1R variants with melanoma risk persisted among those with darker com-

plexion, but was absent among fair-skinned individuals in Australian Caucasians. A similar finding was observed in a Mediterranean population.[19] Similarly, we found that the effect of carrying 1 NRHC allele and 1 RHC allele was strongest among women with medium or olive skin color for melanoma. In contrast, there was a joint effect of red hair and carrying at least 1 RHC allele on melanoma risk. We also observed a significant interaction between red hair color and the Arg151Cys on melanoma risk in a multivariate model controlling for other risk factors. The carriage of the 151Cys variant allele predicted additional risk of melanoma among women with red hair, not among women with other hair colors. The 151Cys allele resulted in partial rescue of coat color in transgenic mice, suggesting red hair phenotype may not be the null phenotype for MC1R.[31] The interactions we observed for MC1R variants with hair color need to be replicated, given the small number of women with red hair.

After adjusting for self-reported pigmentary phenotype, the MC1R variants have been found to remain associated with increased risks of melanoma and nonmelanoma skin cancers, which is consistent with the previous findings among Caucasians in European countries and Australia.[38] There are several proposed explanations for our findings. First, self-reported categorical hair color and skin color may leave room for misclassification of individual report of pigmentation and residual confounding in multivariate analyses. Second, the visible phenotype of hair color is not an accurate measure of hair melanin composition. The eumelanin/phaeomelanin ratio displays marked variation among red-haired individuals.[39] The chemically determined eumelanin/phaeomelanin ratio was an indicator of hair melanin composition and UV sensitivity, and was associated with the MC1R genotype.[26] Third, the MC1R gene may alter the risk of skin cancer through mechanisms other than its effect on pigmentation. The immune and inflammatory responses to UV exposure are at least partially mediated *via* the MC1R gene.[40–42] It has been reported that a-MSH, the MC1R ligand, can also suppress melanoma cell proliferation and reduce the adhesion of melanoma cell to extracellular matrix.[43,44] The MC1R variants failed to exert these effects in the response to a-MSH in transfection assays.[45]

In addition, Dwyer *et al*.[46] reported that, in a Caucasian population in Tasmania, Australia, adding MC1R information to risk prediction based on age, sex and cutaneous melanin modestly increased the area under the ROC curve by 0.01 for melanoma, 0.03 for BCC and 0.02 for SCC. We observed a similar modest additive value of MC1R status to the risk prediction model with several known host factors for skin cancer among US Caucasians.

The limitations of the study include self-reported assessment on pigmentation and exposures, which may lead to misclassification. As discussed above, we performed genotyping on the 7 common polymorphisms, which may have modestly misclassified about 3% of the chromosomes as consensus alleles that may actually contain other rare nonsynonymous variants. We plan to replicate our findings with a larger sample size for invasive melanoma cases only by resequencing the *MC1R* gene. There is potential limitation in generalizability of the results in our cohort of nurses, e.g. outdoor occupations are underrepresented. In conclusion, we provided data supporting that carriage of particular MC1R variants conferred both melanoma and nonmelanoma risks, which was independent from self-reported hair color, skin color, and other risk factors. The 151Cys variant in the *MC1R* gene provided additional information on melanoma risk among red-haired women. We also observed a modest improvement on risk prediction by the MC1R status in line with Whiteman and Green's proposal to include the MC1R genotype in a melanoma risk prediction model.[47]

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