

CORRESPONDENCE

Re: MC1R, ASIP, and DNA Repair in Sporadic and Familial Melanoma in a Mediterranean Population

We read with great interest the article by Landi et al. (1) reporting the association of melanocortin-1 receptor gene (MC1R) variants with melanoma risk and progression in sporadic and familial melanoma patients from northeastern Italy. They observed a two- to fourfold increase in risk of both sporadic and familial melanoma among individuals carrying MC1R variant alleles compared with those carrying wild-type MC1R.

We investigated whether MC1R variants were associated with the risk of sporadic cutaneous melanoma in a population from central and northeastern Italy. We recruited 165 sporadic melanoma patients at the Departments of Dermatology of the Universities of L'Aquila, Modena, and Florence, Italy. For each case patient, one control subject was matched by sex, age (within ± 1 year), and residential area (administrative province). The control subjects were recruited from patients who were treated for diseases unrelated to melanoma by the Surgery and Internal Medicine Departments of the same University Hospitals. Both groups included 82 men and 83 women with a median age of 49 years (range: 17–82 years). The study was approved by the local ethical committees, and written informed consent was obtained from all participants. Information on family and medical history, phenotypic risk factors for melanoma (skin type, hair and eye color, number of melanocytic nevi, and the presence of clinically atypical nevi), and UV exposure habits were collected through a standardized questionnaire and skin examination.

Genomic DNA was isolated from peripheral blood using the QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). All participants were genotyped for six single nucleotide polymorphisms (SNPs) of the MC1R gene ("red hair color" [RHC] variants: R151C, R160W and D294H; and "non-red hair color" [NRHC] variants: V60L, V92M, and

R142H) (2) frequently detected in our population (3,4), using the TaqMan SNP Genotyping Assays allelic discrimination method (Applied Biosystems, Foster City, CA, USA). To validate the SNP assay genotypes, 20 samples were randomly selected for sequencing. Results were consistent with genotypic data. Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from univariate conditional logistic regression models. All statistical tests were two-sided.

Genotype frequencies for the six MC1R variants analyzed are shown in Table 1. Consistent with Landi et al.'s results, the V60L variant was the most common variant in our control population with an allele prevalence of 24.0%. Similarly, carriers of one or more RHC variants had a higher melanoma risk (OR = 2.57; 95% CI = 1.32 to 4.94) compared with carriers of the wild-type sequence. Interestingly, separate analysis of individual MC1R variants showed that the increase in melanoma risk was restricted to the R151C (OR = 3.14; 95% CI = 1.34 to 7.36) and D294H (OR = 11.00; 95% CI = 1.42 to 85.2) alleles.

Using multiple correspondence analysis we obtained optimal scale values for the following categorical variables: hair color, eye color, skin type, number of nevi, presence of clinically atypical nevi, and presence of actinic damage. Evaluation of the eigenvalues suggested the presence of two "latent factors": the first was characterized by the grouping of the categories defining a light pigmentation phenotype (i.e., blond/red hair, blue eyes, skin type I–II); and the second was defined by a high nevus density phenotype (presence of clinically atypical nevi, >50 nevi). Given the optimal scores for each of the categorical variables, we calculated a rating for each subject on the two new variables, "pigmentation factor" and "nevi factor." In our control population, the subjects with RHC variants had a higher pigmentation score (Mann–Whitney test, $P = .009$), with higher prevalence observed for skin type I–II (chi-square, $P = .044$) and light brown eye color (chi-square, $P = .012$). A higher prevalence was observed also for control subjects with a higher nevus score (chi-square, $P = .040$). An association with light pigmentation was

Table 1. Association between melanocortin-1 receptor (MC1R) variants and melanoma risk in an Italian population

MC1R variant	Nucleotide change	Genotype	Cases		Controls		OR (95% CI)*
			No.	%	No.	%	
V60L	178G>T	G/G	109	66.1	95	57.6	1.0 (referent)
		G/T	48	29.1	60	36.4	
		T/T	8	4.8	10	6.1	
V92M	274G>A	G/G	147	89.1	153	92.7	1.0 (referent)
		G/A	17	10.3	12	7.3	
		A/A	1	0.6	0	0.0	
R142H	425G>A	G/G	154	93.3	157	95.2	1.0 (referent)
		G/A	11	6.7	8	4.8	
		A/A	0	0.0	0	0.0	
R151C	451C>T	C/C	142	86.1	157	95.2	1.0 (referent)
		C/T	22	13.3	7	4.2	
		T/T	1	0.6	1	0.6	
R160W	478C>T	C/C	153	92.7	154	93.3	1.0 (referent)
		C/T	11	6.7	11	6.7	
		T/T	1	0.6	0	0.0	
D294H	880G>C	G/G	152	92.1	162	98.2	1.0 (referent)
		G/C	13	7.9	3	1.8	
		C/C	0	0.0	0	0.0	
No variant			55	33.3	62	37.6	1.0 (referent)
Any variant			110	66.7	103	63.4	1.20 (0.77 to 1.88)
NRHC variants†			64	38.8	82	49.7	0.84 (0.51 to 1.39)
≥1 RHC variants‡			46	27.9	21	12.7	2.57 (1.32 to 4.94)
Two or more variants			22	13.3	9	5.5	2.67 (1.14 to 6.29)

*Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from univariate conditional logistic regression models. NRHC = non-red hair color; RHC = red hair color.

†Only NRHC/wild type or NRHC/NRHC.

‡At least one RHC variant.

observed for individuals with one or more NRHC variant alleles (Mann-Whitney test, $P = .031$).

Association between MC1R and melanoma thickness was also investigated in our group of melanoma patients. The odds ratios of having a melanoma ≥ 1 mm were 2.38 (95% CI = 1.01 to 5.60) for carriers of any MC1R variant, 2.80 (95% CI = 1.01 to 7.72) for carriers of one or more RHC variants, and 4.79 (95% CI = 1.43 to 16.03) for carriers of two or more variants compared with melanoma patients homozygous for the wild-type sequence. No multiplicative interaction between genotypes and nevi- or pigmentation-related variables was detected by the likelihood ratio test.

In conclusion, our case-control study supports results recently published by the Journal (1). Our data suggest that individuals with MC1R variants are at increased melanoma risk and progression in a Mediterranean population from central and northeastern Italy.

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NOTES

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RESPONSE

We welcomed the correspondence by Fargnoli et al., which confirmed our findings that variant alleles in the melanocortin-1 receptor (MC1R) gene are associated with melanoma risk, with number of nevi, with pigmentation characteristics, and with melanoma thickness in an independent sample of Italian subjects. Overall, the results observed by Fargnoli et al. were remarkably similar to ours and strongly support the evidence of an important role of MC1R in the pathogenesis of melanoma. Indeed, consistency of inference across two or more independent studies is a key factor for interpreting research data (1) and is one of the guidelines used to judge whether an association is causal or not (2).

The slightly different estimates for melanoma risk and pigmentation among subjects carrying any variant or nonred hair color (NRHC) variants could be due to sample variability or differences in the categorization scheme used for pigmentation characteristics (e.g., for eye color). Because MC1R was not directly sequenced by Fargnoli et al., misclassification of MC1R allele status could have occurred, resulting in an underestimation of the odds ratios (ORs). For example, in our case-control population, 14 of 162 melanoma case patients and eight of 167 control subjects carrying any MC1R variant allele would have been misclassified if we had genotyped only the six variant alleles genotyped by Fargnoli et al. The odds ratio estimating the association between any MC1R variant and melanoma risk would have decreased from 2.12 (95% confidence interval [CI] = 1.2 to 3.6, $P = .005$) to 1.73 (95% CI = 1.0 to 2.9, $P = .04$). The effect of this misclassification would have been

larger if we had investigated the association between multiple MC1R variants and melanoma risk.

When we analyzed each individual red hair color variant as presented by Fargnoli et al., R151C was the most strongly associated with melanoma risk in our study (OR = 3.3, 95% CI = 1.7 to 6.2, $P < .0002$), whereas D294H was only moderately associated (OR = 1.9, 95% CI = 0.7 to 5.4, $P < .2$). Of note, only three control subjects had the D294H variant in Fargnoli et al.'s study, resulting in an unstable estimate with large confidence intervals. Finally, we observed no association between R160W and melanoma risk as reported by our colleagues.

Fargnoli et al. observed a statistically significant association between MC1R variants and thickness of melanoma. We find the confirmation of this association particularly important, because we could not differentiate whether the association we observed was due to a delay in melanoma diagnosis or to a more aggressive type of melanoma. The findings reported by Fargnoli et al., those from our study, and that of a recently published report linking MC1R and risk of multiple melanomas in subjects carrying CDKN2A mutations (3), taken together, suggest a possible role of MC1R in melanoma progression or in determining a more aggressive phenotype of melanoma that results in metastases and/or multiple primary melanoma lesions. Additional, larger studies in different populations are needed to determine whether MC1R could be a predictor of melanoma prognosis. The association between MC1R and risk of melanoma or progression of melanoma appears to extend beyond its association with phenotypic pigmentation in our study, as well as in other studies (4,5). This association may involve different pathways, such as DNA repair (6) or signal transduction, and a more in depth understanding of this mechanism is crucial.

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