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ORIGINAL RESEARCH

MC1R variants as melanoma risk factors independent of at-risk phenotypic characteristics: a pooled analysis from the M-SKIP project

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Purpose: Melanoma represents an important public health problem, due to its high case-fatality rate. Identification of individuals at high risk would be of major interest to improve early diagnosis and ultimately survival. The aim of this study was to evaluate whether *MC1R* variants predicted melanoma risk independently of at-risk phenotypic characteristics.

Materials and methods: Data were collected within an international collaboration – the M-SKIP project. The present pooled analysis included data on 3,830 single, primary, sporadic, cutaneous melanoma cases and 2,619 controls from seven previously published case–control studies. All the studies had information on *MC1R* gene variants by sequencing analysis and on hair color, skin phototype, and freckles, ie, the phenotypic characteristics used to define the red hair phenotype.

Results: The presence of any *MC1R* variant was associated with melanoma risk independently of phenotypic characteristics (OR 1.60; 95% CI 1.36–1.88). Inclusion of *MC1R* variants in a risk prediction model increased melanoma predictive accuracy (area under the receiver-operating characteristic curve) by 0.7% over a base clinical model (P=0.002), and 24% of participants were better assessed (net reclassification index 95% CI 20%–30%). Subgroup analysis suggested a possibly stronger role of *MC1R* in melanoma prediction for participants without the red hair phenotype (net reclassification index: 28%) compared to paler skinned participants (15%).

Conclusion: The authors suggest that measuring the *MC1R* genotype might result in a benefit for melanoma prediction. The results could be a valid starting point to guide the development of scientific protocols assessing melanoma risk prediction tools incorporating the *MC1R* genotype.

Keywords: pooled analysis, genetic epidemiology, cutaneous melanoma, melanocortin 1 receptor, pigmentation

Introduction

Incidence rates of malignant cutaneous melanoma (CM) continue to rise in most European countries, whereas in other countries, rates have become rather stable in recent years.[1] CM still represents an important public health problem for its high case-fatality rate,[2] and thus, identification of individuals at high risk of developing melanoma would be of major interest to improve early diagnosis and ultimately survival.

Known risk factors for CM include sun sensitivity, sun exposure, light hair and eye color, high number of melanocytic nevi, atypical nevi, and family history of melanoma.[3–5] Knowledge of risk factors for CM is the basis for the development of risk prediction tools that may improve understanding and decision-making, leading to favorable behavior change and disease prevention.[6–9] In addition to their clinical uses, these tools can assist in planning intervention trials and prevention strategies

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that target particular risk groups.[7–9] Clinical risk prediction models for CM have been previously reviewed:[10] their discrimination ranged from fair to very good (area under the receiver-operating characteristic curve [AUC] 0.62–0.86), comparable with those obtained for other cancers.[10,11] The US Preventive Services Task Force considered the utility of these tools for population-based screening and concluded that the current evidence was insufficient to assess the balance of benefits and harms of visual skin examination by a primary care clinician or patient self-examination to screen for skin cancer of any type in adults.[2,12] An accompanying editorial suggested that the Preventive Services Task Force statement should be viewed as an invitation to the scientific communities "to work together in executing well-designed studies . . . so future recommendations can be of greater public health benefit".[13] Since melanoma seems to be determined by complex interactions among host characteristics, environmental exposure, and genetic factors,[14,15] the inclusion and evaluation of genetic markers in risk models may be warranted and has been considered an important step for further development and testing of prediction tools before they can be used routinely with confidence.[10]

MC1R is the most important gene found to play a role in predisposition to sporadic CM, and its association with CM has been replicated and confirmed by meta-analyses and genome-wide association studies.[16–21] The *MC1R* gene is located on chromosome 16q24.3 and is a key regulator of skin pigmentation.[22] It is highly polymorphic in populations of European origin, with more than 200 coding region variants described to date[23] and a prevalence of any *MC1R* variant of ~60% in healthy controls.[16] Some of these variants have been shown to reduce receptor function,[24–26] result in a quantitative shift of melanin synthesis from eumelanin to phaeomelanin,[27] and determine the red hair (RH) phenotype, characterized by the co-occurrence of fair skin, RH, freckles, and ultraviolet (UV) radiation sensitivity (poor tanning response and solar lentigines).

Previous melanoma risk prediction models have included *MC1R* alongside base clinical risk factors[15,28–31] and reported slight improvement in risk prediction with *MC1R* inclusion. However, because of the strong relationship between *MC1R* and phenotypic characteristics, their joint inclusion in the same model may generate biased estimates if the effect of *MC1R* on CM is mediated mainly by pigmentation. Therefore, before inclusion of *MC1R* in a risk prediction model in addition to phenotypic characteristics, it should be demonstrated that *MC1R* has a direct effect on CM development through biological pathways that are independent of pigmentation. There is some evidence for a wider biological role, as inherited variation at the *MC1R* locus has been reported to be associated with better melanoma survival overall,[32] but to reduce therapeutic benefit from treatment with BRAF inhibitors.[33] A stronger role of *MC1R* variants in increasing melanoma risk in darker pigmented individuals has been suggested,[16,18,34,35] but the extent to which pigmentation and nonpigmentation pathways account for the association between *MC1R* and CM is still not clear.

Therefore, the aims of this study were 1) to decompose the total risk estimate of *MC1R* on CM into two different effects: one due to the nonpigmentation pathway (direct effect) and one due to the pigmentation pathway (indirect effect); and 2) to evaluate whether the inclusion of *MC1R* variants in risk-prediction models increases their ability to predict CM in both the whole population and targeted subgroups of subjects with different phenotypic characteristics.

Materials and methods

Study population

Data were collected within the M-SKIP (melanocortin 1 receptor, skin cancer, and phenotypic characteristics) project, described in detail elsewhere.[36] Briefly, we gathered original individual data from studies on *MC1R* variants and phenotypic characteristics in patients with sporadic CM and nonmelanoma skin cancer and/or in healthy controls. According to familial melanoma definition,[37,38] sporadic melanoma cases were defined as subjects with no more than one first-degree relative or two any-degree relatives with melanoma. Since 2009, of 49 investigators contacted, 38 (78%) agreed to participate and sent their data along with a signed statement declaring that their original study was approved by an ethics committee.

For the purpose of the present study, we excluded all the nonmelanoma skin cancer cases and included seven melanoma case–control studies[18,30,34,39–43] according to inclusion criteria of the *MC1R* gene being sequenced and there being information available on hair color, skin phototype, and freckles, ie, the phenotypic characteristics used to define the RH phenotype. These phenotypic characteristics were those associated with *MC1R* genetic variants in our previous publication.[44] The present study included data on 3,830 CM cases and 2,619 controls (Table 1).

Statistical analysis

A complete description of statistical analysis methods is reported in the Supplementary material.

Table 1 Description of the studies included in the analysis

Study	Country	Cases	Controls	Control type[a]	RH phenotype[b] in controls	Available confounders[c]
Kennedy et al[39]	The Netherlands	115	377	Hospital	210 (56%)	Sun exposure, sunburn, common and atypical nevi
Landi et al[34]	Italy	163	169	Healthy	83 (49%)	Sun exposure, sunburn, common nevi
Bishop et al[40]	UK	1567	469	Hospital	314 (67%)	Sunburn[d]
Kanetsky et al[18]	USA	766	322	Healthy	262 (81%)	Sun exposure, sunburn, atypical nevi[e]
Menin et al[41,f]	Italy	118	168	Healthy	70 (42%)	Sunburn, common and atypical nevi
Ghiorzo et al[42]	Italy	236	355	Healthy	224 (63%)	Sunburn[d]
Penn et al[30]	USA	865	759	Healthy	339 (45%)	Sun exposure, sunburn, common nevi
Total		3,830	2,619		1,502 (57%)	

Notes: [a]Healthy controls were population controls, friends or partners of cases, outpatients, or hospital personnel. [b]Defined as presence of red hair, freckles, or skin type I/II; [c]Beyond age and sex, which were available in all seven studies. Confounders with more than 20% of missing data not listed. Sun exposure includes separate information on chronic and intermittent sun exposure. [d]Information on atypical nevi was also available, but with more than 20% of subjects with missing data. [e]Not included in risk model analysis because of missing data on common nevi. [f]Included an unpublished group of sporadic melanoma cases that were included in the present analysis. Study approach, control group, and genetic analysis were the same as described in Menin et al.[41]

Abbreviation: RH, red hair.

Mediation analysis

To estimate the independent contribution of *MC1R* variants on CM development, we performed a mediation analysis.[45,46] We decomposed the overall risk estimate for CM associated with *MC1R* into a direct effect due to the nonpigmentation pathway and an indirect effect due to the pigmentation pathway. We estimated the direct effect of *MC1R* (any variant and the nine single common variants vs wild type [WT] on CM in the presence and in the absence of the RH phenotype (controlled direct effect [CDE]). Following our previous publication,[44] RH phenotype was primarily defined as the presence of at least one of the characteristics of RH, freckles, and skin type I/II. Skin type is a measure of sun sensitivity of the skin and was defined in our study according to the known Fitzpatrick classification as type I (always burns, never tans), II (usually burns, tans minimally), III (sometimes mild burns, tans uniformly), and IV (never burns, tans easily). We also estimated the natural direct effect (NDE), which essentially averages CDE over the population and finally the indirect effect of *MC1R* mediated by RH phenotype (natural indirect effect [NIE]). Mediation analysis was separately applied to each of the seven studies, and ORs with 95% CIs were obtained for total effect (TE), NDE, NIE, and CDE using unconditional logistic regression models with the following covariates (when available) of age, sex, intermittent and chronic sun exposure, lifetime and childhood sunburns, family history of melanoma, common nevi count, and presence of atypical nevi. Following the two-stage analysis approach, we pooled study-specific ORs with a random effects model. We calculated I^2-values to assess the percentage of total variation across studies that was attributable to heterogeneity rather than to chance.

Model comparison

We tested the prediction ability to identify CM participants by adding *MC1R* variants to a clinical base prediction model. Variables included in the base model were age, sex, sunburn, number of common nevi, and RH phenotype. These covariates were available in a subset of 4,390 (68%) participants from six studies. We used unconditional logistic regression to estimate the risk of CM according to the base clinical risk model and to the model including the *MC1R* gene, defined as the presence of any *MC1R* variants versus WT, the presence of only r variants and presence of at least one R variant versus WT, and the presence of each of the nine most common *MC1R* variants or rarer variants. R and r alleles have previously been defined according to their association with RH phenotype.[17,22] We compared the predictive ability of the model with *MC1R* over the base clinical model by receiver-operating characteristic (ROC) curves, net reclassification improvement (NRI), and decision curve analysis. Analysis was carried out with the software SAS (version 9.2) and Stata (version 11.2).

Results

The main characteristics of the studies included are summarized in Table 1. Three studies were performed in Italy, two in the US, one in the UK, and one in the Netherlands. All studies included more than 97% Caucasians. Two studies included hospital-based controls,[30,31] and five recruited healthy controls. One study[41] included an unpublished group of sporadic melanoma cases. The study approach, control group, and genetic analysis were the same described in the corresponding published paper.

Direct and indirect effects of *MC1R* on CM development

The OR (95% CI) for the TE of any *MC1R* variants on CM risk was 1.71 (1.46–2.00; I^2=0; Figure 1). When decomposed, the risk was primarily due to the NDE, independent of phenotypic characteristics (OR 1.60; 95% CI 1.36–1.88; I^2=0; Figure 1); the NIE, which would be dependent on the pigmentation pathway, was smaller (OR 1.07; 95% CI 1.03–1.11; I^2=0; Figure 1). When the CDE according to RH phenotype was examined, we found a direct, positive association between *MC1R* and CM in the absence of RH phenotype (OR 1.75; 95% CI 1.33–2.33; I^2=0; Figure 2) and a smaller direct association between *MC1R* and CM in participants with the RH phenotype (OR 1.50; 95% CI 1.19–1.89; I^2=37%; Figure 2).

Looking at each of the nine most common *MC1R* variants (Table S1), we still found for all of them larger NDE than NIE, with significant NDE found for the R variants R142H, R151C, R160W, and D294K (ranging from OR 2.22; 95% CI 1.33–3.71 to OR 3.55; 95% CI 1.21–10.47) and significant NIE found only for the R variant R151C (OR 1.18; 95% CI 1.00–1.39). Furthermore, CDE was higher for non-RH-phenotype subjects than for RH-phenotype subjects for the most common variants (allele frequency ≥1.5%), while it was opposite for the three rarer *MC1R* variants D84E, R142H, and I155T (Table S1).

Risk models for CM prediction

Table 2 reports the ORs and 95% CIs for variables included in the base clinical risk model and for *MC1R* variants. Having more than 30 common nevi and RH phenotype increased CM risk in our population (Table 2). Independent of other risk factors, carriers of any *MC1R* variant had a higher risk of CM than noncarriers (OR 1.63; 95% CI 1.40–1.90). The OR slightly decreased when the analysis was restricted to RH participants, while it increased for non-RH participants (Table 2). When we considered a distinction between *MC1R* r and R variants, in comparison with WT, carriers of at least one R variant had a higher risk of CM (OR 2.08; 95% CI 1.76–2.46) than carriers of only r variants (OR 1.24; 95% CI 1.04–1.47). For RH participants, carrying only *MC1R* r variants did not increase CM risk, while the risk was increased for carriers of *MC1R* R variants. By contrast, both *MC1R* r and R variants were associated with a higher risk of CM in participants with the non-RH phenotype (Table 2). Similar results were found looking at each of the nine *MC1R* variants separately (Table S2).

The clinical risk model yielded an AUC of 0.706 (95% CI 0.691–0.721; Table S3). The model including any *MC1R* variant showed slightly greater discrimination, with an AUC of 0.713 (95% CI 0.698–0.728; *P*=0.002) and an NRI of 24% (95% CI 20%–30%). Differentiation between r and R variants and considering each single variant further increased diagnostic accuracy by 1.5% and 1.9%, respectively, over the base clinical risk model, with an NRI of 37% (95% CI 32%–43%) and 34% (28%–39%), respectively. Subgroup analysis restricted to participants with the non-RH phenotype revealed that *MC1R* improved the AUC by 1.8% (from 0.678 to 0.696, *P*=0.0008; Figure 3; Table S3), suggesting a stronger role of *MC1R* in melanoma prediction for darker pigmented participants compared to RH participants. The NRI due to *MC1R* inclusion for participants with a non-RH phenotype was 28% (95% CI 19%–37%), while it was 15% (95% CI 9%–22%) for RH participants. The addiction of separate information on r and R *MC1R* variants and on single specific variants obtained a better model performance for both RH and non-RH participants. Decision curves showed a small increase in net benefit of *MC1R* testing for non-RH participants over almost the entire range of threshold probabilities (Figure S1), with an average increase in net benefit of 0.003 for the model with any *MC1R* variant and 0.005 for the model with r or R *MC1R* variant over the base clinical model.

Sensitivity analysis on a subset of 2,472 (38%) participants from four studies with additional information on atypical nevi provided similar results (not shown): having more than 30 common nevi, RH phenotype, and atypical nevi increased CM risk. In this sensitivity analysis, *MC1R* variants increased CM risk in non-RH participants, but not in RH participants. Sensitivity analysis with different definitions of RH phenotype provided similar results (not shown).

Discussion

Our pooled analysis showed that the presence of any *MC1R* variant had a direct effect on CM, conferring a 60% higher risk to carriers versus noncarriers. The pigmentation-mediated effect of *MC1R* on CM was smaller with any *MC1R* variant and each of the nine most common *MC1R* variants. This result confirms and expands the previous suggestion[16–18,34] of the existence of a nonpigmentation pathway leading *MC1R* to CM development. Here, we give for the first time an estimate of the magnitude of total effect explained by each of the two (pigmentation and nonpigmentation) pathways. Recent studies and reviews[47] have implicated *MC1R* signaling in a number of key biological pathways

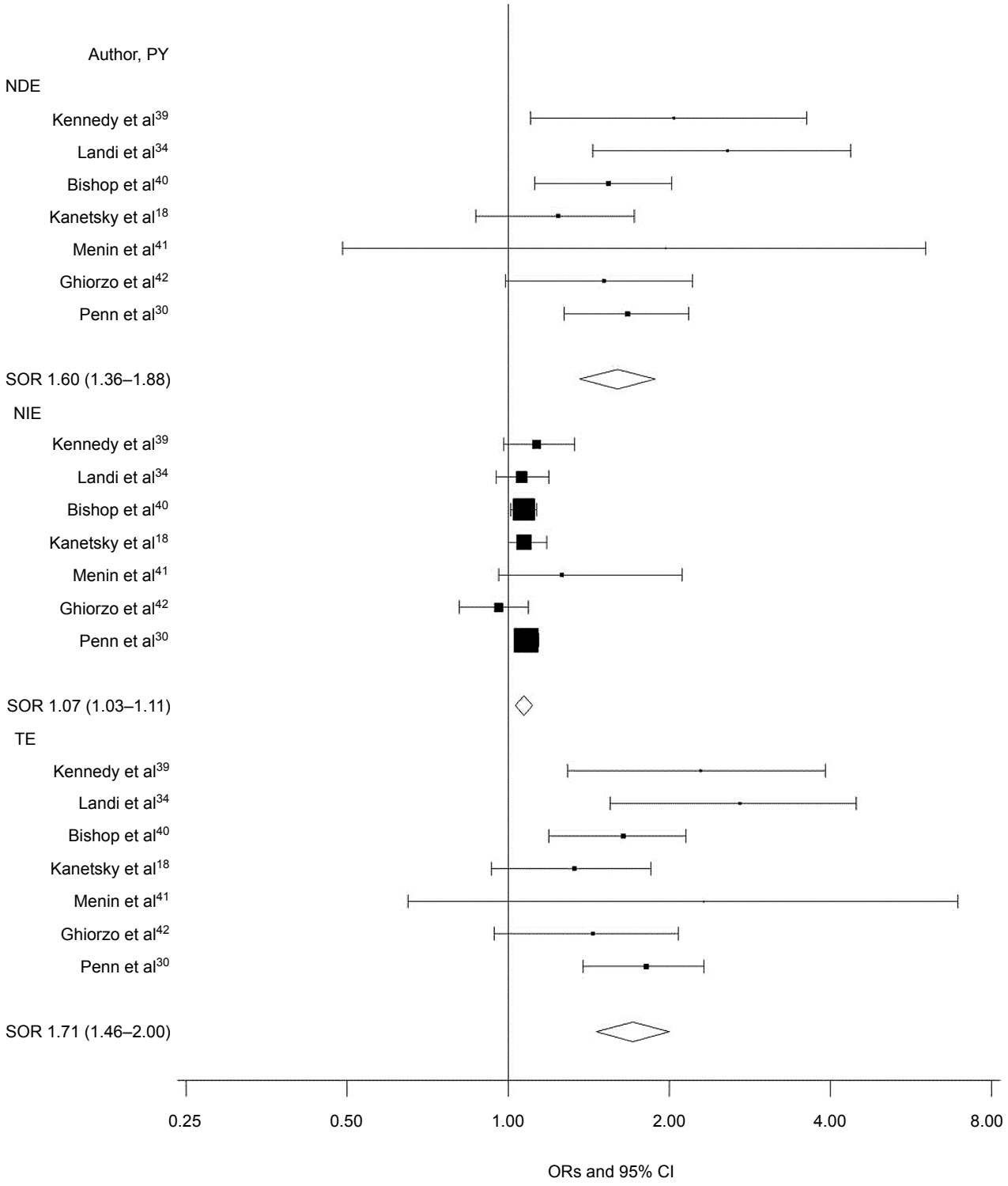

Figure 1 Forest plot for NDE, NIE, and TE of any *MC1R* variant on melanoma risk.
Notes: CDE estimates the direct effect of *MC1R* on melanoma when the mediator is controlled at level 0 (absent) or 1 (present) uniformly in the population, NDE essentially averages CDE over the population, NIE estimates the indirect effect of *MC1R* mediated by RH phenotype, and TE is the overall melanoma risk estimate for *MC1R* carriers and in each study is the product of NDE and NIE.
Abbreviations: CDE, control direct effect; NDE, natural direct effect; NIE, natural indirect effect; PY, publication year; RH, red hair; SOR, summary OR; TE, total effect.

involved in cell-cycle control,[48] apoptosis,[49] and activation of DNA-repair mechanisms and antioxidant defenses.[50] Production of pheomelanin pigments seems associated with increased oxidative DNA damage compared with synthesis of eumelanins.[51] Further evidence for pheomelanin-associated increased cellular oxidative stress was obtained in studies of

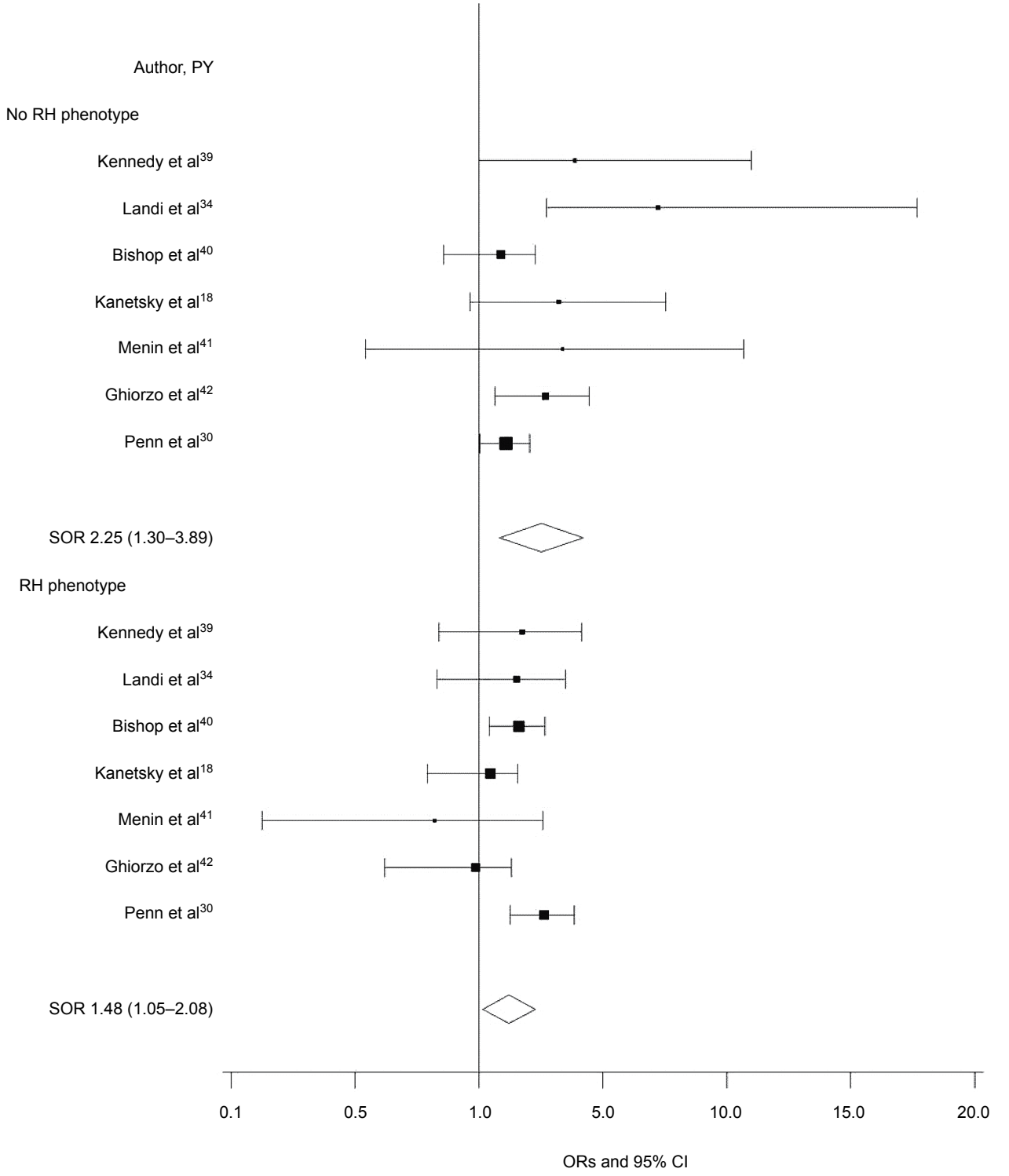

Figure 2 Forest plot for control direct effect of any *MC1R* variant on melanoma risk according to RH phenotype.*
Notes: *Defined as presence of red hair, freckles, or skin type I/II. Control direct effect estimates the direct effect of *MC1R* on melanoma when the mediator is controlled at level 0 (absent) or 1 (present) uniformly in the population.
Abbreviations: PY, publication year; RH, red hair; SOR, summary OR.

mice carrying a loss-of-function mutation of the *Mc1r* gene, which provided evidence in support that the pheomelanin-pigment pathway produces UV-independent carcinogenic contributions to melanomagenesis.[52] Another recent study[53] found a role of germ-line *MC1R* variants in influencing the somatic mutational landscape of melanoma, with an expected

Table 2 ORs with 95% CIs for melanoma risk according to a base clinical model and the same model with inclusion of MC1R variants

	All participants (n=4,390)		RH participants (n=2,654)		Non-RH participants (n=1,736)	
	Base model	Base model + MC1R	Base model	Base model + MC1R	Base model	Base model + MC1R
Age[a]	0.97 (0.94–1.00)	0.97 (0.94–1.00)	**0.96 (0.92–0.99)**	**0.96 (0.92–0.99)**	0.99 (0.94–1.04)	0.99 (0.94–1.04)
Sex						
Male	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Female	1.01 (0.89–1.16)	1.03 (0.91–1.18)	0.92 (0.77–1.09)	0.92 (0.77–1.10)	1.18 (0.96–1.45)	1.23 (1.00–1.52)
Sunburn						
None	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Any	1.15 (0.98–1.35)	1.11 (0.94–1.30)	1.16 (0.94–1.43)	1.13 (0.91–1.39)	1.13 (0.88–1.47)	1.08 (0.84–1.41)
Common nevi						
≤30	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
>30	**3.37 (2.90–3.92)**	**3.40 (2.92–3.96)**	**3.46 (2.86–4.18)**	**3.47 (2.87–4.20)**	**3.25 (2.53–4.16)**	**3.30 (2.57–4.24)**
Phenotype						
Non-RH	1.00 (reference)	1.00 (reference)	–	–	–	–
RH	**1.64 (1.43–1.88)**	**1.52 (1.32–1.75)**	–	–	–	–
MC1R						
None	–	1.00 (reference)	–	1.00 (reference)	–	1.00 (reference)
Any variant	–	**1.63 (1.40–1.90)**	–	**1.55 (1.25–1.92)**	–	**1.76 (1.41–2.19)**
Only r variants	–	**1.24 (1.04–1.47)**	–	1.07 (0.84–1.37)	–	**1.45 (1.14–1.86)**
≥1 R variant	–	**2.08 (1.76–2.46)**	–	**1.92 (1.53–2.41)**	–	**2.25 (1.73–2.92)**

Notes: [a]Per 5-year increase. Significant ORs are in bold. All models are adjusted for variables included in the table + study center. Two separate models were created for 1) any MC1R variant vs wild type and 2) only r variants and ≥1R variant vs wild type. R and r alleles were defined basing on their stronger or weaker association with the RH phenotype for the most common variants[44,67–70] and on likely pathogenicity using the algorithm proposed by Davies et al[32] for the less common variants.
Abbreviation: RH, red hair.

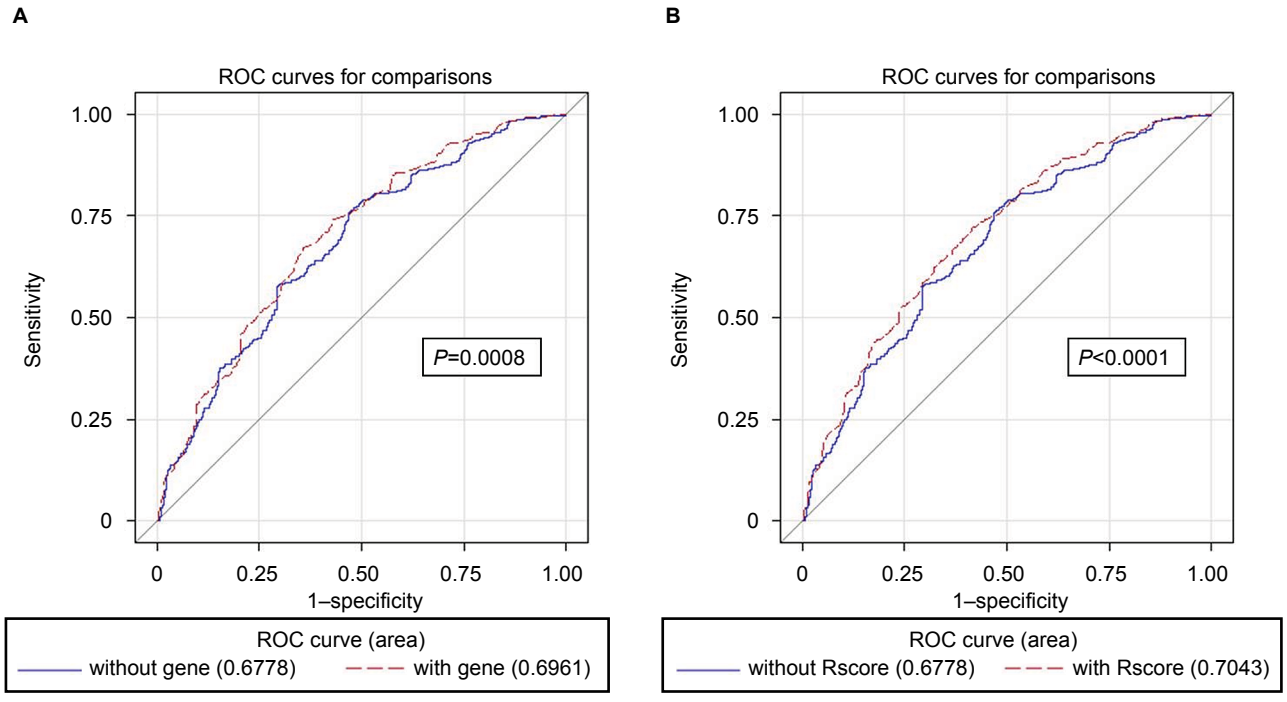

Figure 3 ROC curve comparison between base clinical model and the same model with inclusion of *MC1R* variants for patients with no RH phenotype.*
Notes: (**A**) *MC1R* defined as the presence or absence of any *MC1R* variant and (**B**) as no *MC1R* variant, only r variants, and ≥1 R variants. *Non-RH patients defined as those without RH and freckles and with skin type III/IV. R and r alleles were respectively defined basing on their stronger or weaker association with the RH phenotype for the most common variants[44,67–70] and on likely pathogenicity using the algorithm proposed by Davies et al[32] for the less common variants.
Abbreviations: RH, red hair; ROC, receiver-operating characteristic.

higher number of somatic C>T mutations in carriers of R alleles than those without R alleles. In this respect, it is worth noting that although the most relevant UV radiation-induced mutations are C>T transitions, highly recurrent mutations in key melanoma-driver genes, such as the V600E mutation in *BRAF*, are non-C>T changes. Importantly, significant increases in the rate of non-C>T changes, some of which might depend on oxidative DNA damage, have also been found in R allele carriers compared with noncarriers.[53,54] Accordingly, associations of *MC1R* and genes frequently mutated in melanoma, such as *BRAF* or *TERT*, have been reported.[55–57]

We found that *MC1R* slightly improved risk prediction accuracy over a base clinical model, especially for non-RH participants: CM predictive accuracy increased by 1.8% and the CM risk of 28% of participants was better assessed. If a distinction is used in the model to differently score r and R variants, the benefit for the whole population increased from 24% of participants correctly reclassified with just presence/absence of *MC1R* variants to 37% of participants correctly reclassified with separate information on r and R variants. Distinction between r and R alleles, however, was more apparent for RH than for non-RH participants. In the study by Cust et al,[15] the R variants were responsible for most of the improvement in risk prediction, but separate analysis for RH and non-RH participants was not performed.

Previous melanoma risk prediction models have included *MC1R* with base clinical risk factors.[15,28–30] Whiteman and Green[28] did not report on predictive ability. Stefanaki et al[29] found no improvement in AUC with the addition of eight melanoma-associated single-nucleotide polymorphisms (SNPs) to the base model. Both Cust et al[15] and Penn et al[30] reported slight improvement in AUC with the inclusion of *MC1R*. However, no previous paper has reported separate results according to fairer or darker phenotypic characteristics. This point seems in fact extremely important, because *MC1R* seems to have a stronger role in non-RH participants in both the present paper and in previously published stratified analyses.[16,18,34] A more precise risk assessment, therefore, in participants with no RH, no freckles, and skin type III/IV could potentially change individual clinical follow-up schedules and perhaps UV-exposure behavior and indoor tanning habits.

The application of risk prediction tools in cancer screening has been widely discussed. In particular, there have been concerns on the impact of genetic screening in clinical decision-making. For example, in a previous review,[58] genetic screening was discussed using commercially available SNP panel tests in prostate cancer. Conclusions were that the investigated SNP panels had poor discriminative ability and clinical validity. In our study, adding the *MC1R* genotype resulted in a small yet significant improvement in predictive ability over the clinical model and a substantial change in the NRI, and it is worth noting that this improvement was based on a single gene, while risk indices for both prostate and breast cancer require several genetic markers to produce increases of similar magnitude.[59–62] Decreasing genotyping costs and increasing use of genetic testing is making it more feasible to incorporate genetic risk factors into clinical risk prediction tools, and limiting testing to the non-RH participants with no other risk factors may result in a cost-effective strategy via better allocation of resources. However, translation into routine clinical practice requires several additional steps,[63,64] and new studies are needed in order better to assess the clinical utility of these models, taking also into account the small increase in net benefit observed in our decision curve analysis.

Our study has several strengths. We quantified for the first time the amount of total effect of *MC1R* on CM due to pigmentation and nonpigmentation pathways. Previous stratified analyses, including ours,[16] have already suggested that the effect of *MC1R* was stronger in darker pigmented participants; however, stratified analyses are not conclusive, especially in the presence of genotype–phenotype interaction.[46,65] Precise and powerful quantification of the effect of the two pathways was only feasible in the present analysis after inclusion of new studies.[30,40,41] The large sample and international collaborative nature of the M-SKIP project make it possible to assess various populations and ancestries, thus providing results that are robust and consistent in different geographical areas. We were also able to create different predictive models according to the RH and non-RH phenotypes, which was not possible in previous studies. In our centralized statistical analysis, we were able to take into account all the available confounders, with a homogeneous plan of analysis and definition of covariables.

Heterogeneity among different populations is a possible limitation of our study; therefore, this tool may require adjustments before being applicable to each specific population.[10] However, it is not easy to develop a good and precise tool for each population due to the lack of power of single studies. Moreover, we did not observe any heterogeneity in risk estimates for *MC1R* and CM, suggesting that information on *MC1R* improves CM risk prediction in different populations of European origins. Following our previous publication,[44] RH participants were defined as participants with either

RH, freckles, or skin type I/II, and we are aware that other definitions may modify the results. However, in a sensitivity analysis using RH defined as a score obtained from multiple correspondence analysis,[44] the results were similar. Phenotype misclassification is a possibility, although a previous study reported a good correlation between self-defined skin pigmentation and measured melanin density.[66] In order to minimize phenotype misclassification, we performed a sensitivity analysis that included only extreme categories of the RH phenotype.[5] Although this analysis was underpowered, we observed similar risk estimates to those reported for the main analysis in the present paper (results not shown). Finally, it should be noted that our analyses were performed on sporadic melanoma cases, and thus, generalization to familial melanoma is not appropriate.

Conclusion

We found a direct role of *MC1R* in melanoma risk independently of RH phenotype and demonstrated that adding the *MC1R* genotype to classical clinical risk factors results in a benefit for CM prediction. A change in clinical follow-up schedules and UV exposure and sun protection habits of identified at-risk individuals might favor early melanoma diagnosis and prevention. The application of risk prediction tools in cancer screening has been controversial, because of concerns on their impact in clinical decision-making. Our results could be a valid starting point to guide the development of scientific protocols assessing melanoma risk prediction tools incorporating the *MC1R* genotype, ideally with a prospective design and cost–benefit evaluation.

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Disclosure

The authors report no conflicts of interest in this work.

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