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

Elena Tagliabue,¹ Sara Gandini,² Rino Bellocco,^{3,4} Patrick Maisonneuve,² Julia Newton-Bishop,⁵ David Polsky,⁶ DeAnn Lazovich,⁷ Peter A Kanetsky,⁸ Paola Ghiorzo,^{9,10} Nelleke A Gruis,¹¹ Maria Teresa Landi,¹² Chiara Menin,¹³ Maria Concetta Fargnoli,¹⁴ Jose Carlos García-Borrón,^{15,16} Jiali Han,¹⁷ Julian Little,¹⁸ Francesco Sera,¹⁹ Sara Raimondi²

On behalf of the M-SKIP Study Group

¹Clinical Trial Center, Scientific Directorate, Fondazione IRCCS Istituto Nazionale dei Tumori, ²Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy; ³Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; ⁴Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy; ⁵Section of Epidemiology and Biostatistics, Institute of Cancer and Pathology, University of Leeds, Leeds, UK; ⁶Ronald O. Perleman Department of Dermatology, New York University School of Medicine, NYU Langone Medical Center, New York, NY; ⁷Division of Epidemiology and Community Health, University of Minnesota, MN; ⁸Department of Cancer Epidemiology, H Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; ⁹Department of Internal Medicine and Medical Specialties, University of Genoa, ¹⁰IRCCS AOU San Martino-IST, Genoa, Italy; ¹¹Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands; ¹²Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD, USA; ¹³Immunology and Molecular Oncology Unit, Veneto Institute of Oncology, IOV-IRCCS, Padua; ¹⁴Department of Dermatology, University of L'Aquila, L'Aquila, Italy; ¹⁵Department of Biochemistry, Molecular Biology, and Immunology, University of Murcia, ¹⁶IMIB-Arrixaca, Murcia, Spain; ¹⁷Department of Epidemiology, Richard M Fairbanks School of Public Health, Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, IN, USA; ¹⁸School of Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada; ¹⁹Department of Social and Environmental Health Research, London School of Hygiene and Tropical Medicine, London, UK

Correspondence: Sara Raimondi
Division of Epidemiology and Biostatistics, European Institute of Oncology, 16 Via Adamello, Milan 20139, Italy
Tel +39 02 9437 2711
Fax +39 02 5748 9922
Email sara.raimondi@ieo.it

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

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 date²³ 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 pheomelanin,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: ^aHealthy controls were population controls, friends or partners of cases, outpatients, or hospital personnel. ^bDefined as presence of red hair, freckles, or skin type I/II; 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. ^cInformation on atypical nevi was also available, but with more than 20% of subjects with missing data. ^dNot included in risk model analysis because of missing data on common nevi. ^eIncluded 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 study⁴¹ 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; $P=0$; 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; $P=0$; Figure 1); the NIE, which would be dependent on the pigmentation pathway, was smaller (OR 1.07; 95% CI 1.03–1.11; $P=0$; 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; $P=0$; 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; $P=37\%$; 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 $\geq 1.5\%$), 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 addition 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 reviews⁴⁷ 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 *Mclr* gene, which provided evidence in support that the pheomelanin-pigment pathway produces UV-independent carcinogenic

contributions to melanomagenesis.⁵² Another recent study⁵³ 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 MCIR variants

	All participants (n=4,390)		RH participants (n=2,654)		Non-RH participants (n=1,736)	
	Base model	Base model + MCIR	Base model	Base model + MCIR	Base model	Base model + MCIR
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)	–	–	–	–
MCIR						
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: ^aPer 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 MCIR variant vs wild type and 2) only r variants and ≥1 R 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 al³² for the less common variants.

Abbreviation: RH, red hair.

A

B

Figure 3 ROC curve comparison between base clinical model and the same model with inclusion of MCIR variants for patients with no RH phenotype.*

Notes: (A) MCIR defined as the presence or absence of any MCIR variant and (B) as no MCIR 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 al³² 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 Green²⁸ did not report on predictive ability. Stefanaki et al²⁹ found no improvement in AUC with the addition of eight melanoma-associated single-nucleotide polymorphisms (SNPs) to the base model. Both Cust et al¹⁵ and Penn et al³⁰ 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.

Acknowledgments

This work was supported by the Italian Association for Cancer Research (grant MFAG 11831). The Melanoma Susceptibility Study (PAK) was supported by the National Cancer Institute (CA75434, CA80700, CA092428). The Genoa study (PG) was supported by AIRC IG 15460. The M-SKIP study group consists of the following members: principal investigator (PI), Sara Raimondi (European Institute of Oncology, Milan, Italy); advisory committee members, Philippe Autier (International Prevention Research Institute, Lyon, France), Maria Concetta Fagnoli (University of L'Aquila, Italy), José C García-Borrón (University of Murcia, Spain), Jiali Han (Indiana University, Indianapolis, IN, USA), Peter A Kanetsky (Department of Cancer Epidemiology, H Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA), Maria Teresa Landi (National

Cancer Institute, NIH, Bethesda, MD, USA), Julian Little (University of Ottawa, Canada), Julia Newton-Bishop (University of Leeds, Leeds, UK), and Francesco Sera (London School of Hygiene and Tropical Medicine, London, UK); consultants, Saverio Caini (ISPO, Florence, Italy), Sara Gandini, and Patrick Maisonneuve (European Institute of Oncology, Milan, Italy); participant investigators, Albert Hofman, Manfred Kayser, Fan Liu, Tamar Nijsten, and Andre G Uitterlinden (Erasmus MC University Medical Center, Rotterdam, Netherlands), Rajiv Kumar (German Cancer Research Center, Heidelberg, Germany), Tim Bishop, Faye Elliott (University of Leeds, Leeds, UK), Eduardo Nagore (Instituto Valenciano de Oncología, Valencia, Spain), DeAnn Lazovich (Division of Epidemiology and Community Health, University of Minnesota, MN, USA), David Polsky (New York University School of Medicine, New York, NY, USA), Johan Hansson, Veronica Hoiom (Karolinska Institutet, Stockholm, Sweden), Paola Ghiorzo, Lorenza Pastorino (University of Genoa, Italy), Nelleke A Gruis, Jan Nico Bouwes Bavinck (Leiden University Medical Center, Leiden, Netherlands), Ricardo Fernandez-de-Misa (Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain), Paula Aguilera, Celia Badenas, Cristina Carrera, Pol Gimenez-Xavier, Josep Malvehy, Miriam Potrony, Susana Puig, Joan Anton Puig-Butille, Gemma Tell-Marti (Hospital Clinic, IDIBAPS, and CIBERER, Barcelona, Spain), Terence Dwyer (Murdoch Children's Research Institute, Melbourne, Australia), Leigh Blizzard, Jennifer Cochrane (Menzies Institute for Medical Research, Hobart, Australia), Wojciech Branicki (Institute of Forensic Research, Krakow, Poland), Tadeusz Debniak (Pomeranian Medical University, Szczecin, Poland), Niels Morling, Peter Johansen (University of Copenhagen, Copenhagen, Denmark), Susan Mayne, Allen Bale, Brenda Cartmel, Leah Ferrucci (Yale School of Public Health and Medicine, New Haven, CT, USA), Ruth Pfeiffer (National Cancer Institute, NIH, Bethesda, MD, USA), Giuseppe Palmieri (Istituto di Chimica Biomolecolare, CNR, Sassari, Italy), Gloria Ribas (Fundación Investigación Clínico de Valencia Instituto de Investigación Sanitaria, INCLIVA, Spain), Chiara Menin (Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy), Alexander Stratigos, Katerina Kypreou (Andreas Sygros Hospital, University of Athens, Athens, Greece), Anne Bowcock, Lynn Cornelius, M Laurin Council (Washington University School of Medicine, St Louis, MO, USA), Tomonori Motokawa (POLA Chemical Industries, Yokohama, Japan), Sumiko Anno (Shibaura Institute of Technology, Tokyo, Japan), Per Helsing, Per Arne Andresen (Oslo University

Hospital, Oslo, Norway), Gabriella Guida, Stefania Guida (University of Bari, Bari, Italy), Terence H Wong (University of Edinburgh, Edinburgh, UK), and the GEM study group. Participants in the GEM study group are as follows: coordinating center, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, Marianne Berwick (PI, currently at the University of New Mexico), Colin Begg (co-PI), Irene Orlow (coinvestigator), Urvi Mujumdar (project coordinator), Amanda Hummer (biostatistician), Klaus Busam (dermatopathologist), Pampa Roy (laboratory technician), Rebecca Canchola (laboratory technician), Brian Clas (laboratory technician), Javiar Cotignola (laboratory technician), and Yvette Monroe (interviewer); study centers; University of Sydney and Cancer Council New South Wales, Sydney (Australia), Bruce Armstrong (PI), Anne Krickler (co-PI), Melisa Litchfield (study coordinator); Menzies Institute for Medical Research, University of Tasmania, Hobart (Australia), Terence Dwyer (PI), Paul Tucker (dermatopathologist), Nicola Stephens (study coordinator); British Columbia Cancer Agency, Vancouver, BC (Canada), Richard Gallagher (PI), Teresa Switzer (coordinator), Cancer Care Ontario, Toronto, ON (Canada), Loraine Marrett (PI), Beth Theis (coinvestigator), Lynn From (dermatopathologist), Noori Chowdhury (coordinator), Louise Vanasse (coordinator), Mark Purdue (research officer), David Northrup (manager for CATI), Centro per la Prevenzione Oncologia Torino, Piedmont (Italy), Roberto Zanetti (PI), Stefano Rosso (data manager), Carlotta Sacerdote (coordinator); University of California, Irvine, CA (USA), Hoda Anton-Culver (PI), Nancy Leighton (coordinator), Maureen Gildea (data manager); University of Michigan, Ann Arbor, MI (USA), Stephen Gruber (PI), Joe Bonner (data manager), Joanne Jeter (Coordinator); New Jersey Department of Health and Senior Services, Trenton, NJ (USA), Judith Klotz (PI), Homer Wilcox (co-PI), Helen Weiss (coordinator); University of North Carolina, Chapel Hill, NC (USA), Robert Millikan (PI), Nancy Thomas (coinvestigator), Dianne Mattingly (coordinator), Jon Player (laboratory technician), Chiu-Kit Tse (data analyst); University of Pennsylvania, Philadelphia, PA (USA), Timothy Rebbeck (PI), Peter Kanetsky (coinvestigator), Amy Walker (laboratory technician), Saarene Panossian (laboratory technician); consultants, Harvey Mohrenweiser, University of California, Irvine, Irvine, CA (USA); Richard Setlow, Brookhaven National Laboratory, Upton, NY (USA).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Erdmann F, Lortet-Tieulent J, Schuz J, et al. International trends in the incidence of malignant melanoma 1953–2008: are recent generations at higher or lower risk? *Int J Cancer*. 2013;132(2):385–400.
2. Wernli KJ, Henrikson NB, Morrison CC, Nguyen M, Pocobelli G, Blasi PR. Screening for skin cancer in adults: updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA*. 2016;316(4):436–447.
3. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma – I: common and atypical naevi. *Eur J Cancer*. 2005;41(1):28–44.
4. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma – II: sun exposure. *Eur J Cancer*. 2005;41(1):45–60.
5. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma – III: family history, actinic damage and phenotypic factors. *Eur J Cancer*. 2005;41(14):2040–2059.
6. Ahmed H, Naik G, Willoughby H, Edwards AG. Communicating risk. *BMJ*. 2012;344:e3996.
7. Freedman AN, Seminara D, Gail MH, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. *J Natl Cancer Inst*. 2005;97(10):715–723.
8. Jackson A, Wilkinson C, Ranger M, Pill R, August P. Can primary prevention or selective screening for melanoma be more precisely targeted through general practice? A prospective study to validate a self administered risk score. *BMJ*. 1998;316(7124):34–39.
9. Quereux G, N’Guyen JM, Cary M, Jumbou O, Lequeux Y, Dreno B. Validation of the self-assessment of melanoma risk score for a melanoma-targeted screening. *Eur J Cancer Prev*. 2012;21(6):588–595.
10. Vuong K, McGeechan K, Armstrong BK, Cust AE. Risk prediction models for incident primary cutaneous melanoma: a systematic review. *JAMA Dermatol*. 2014;150(4):434–444.
11. Olsen CM, Neale RE, Green AC, et al. Independent validation of six melanoma risk prediction models. *J Invest Dermatol*. 2015;135(5):1377–1384.
12. US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, et al. Screening for skin cancer: US preventive services task force recommendation statement. *JAMA*. 2016;316(4):429–435.
13. Tsao H, Weinstock MA. Visual inspection and the US Preventive Services Task Force recommendation on skin cancer screening. *JAMA*. 2016;316(4):398–400.
14. Chaudru V, Chompret A, Bressac-de Paillerets B, Spatz A, Avril MF, Demenais F. Influence of genes, nevi, and sun sensitivity on melanoma risk in a family sample unselected by family history and in melanoma-prone families. *J Natl Cancer Inst*. 2004;96(10):785–795.
15. Cust AE, Goumas C, Vuong K, et al. MC1R genotype as a predictor of early-onset melanoma, compared with self-reported and physician-measured traditional risk factors: an Australian case-control-family study. *BMC Cancer*. 2013;13:406.
16. Pasquali E, Garcia-Borrón JC, Fargnoli MC, et al. MC1R variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project. *Int J Cancer*. 2015;136(3):618–631.
17. Raimondi S, Sera F, Gandini S, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer*. 2008;122(12):2753–2760.
18. Kanetsky PA, Panossian S, Elder DE, et al. Does MC1R genotype convey information about melanoma risk beyond risk phenotypes? *Cancer*. 2010;116(10):2416–2428.
19. Chatzinasiou F, Lill CM, Kypreou K, et al. Comprehensive field synopsis and systematic meta-analyses of genetic association studies in cutaneous melanoma. *J Natl Cancer Inst*. 2011;103(16):1227–1235.
20. Amos CI, Wang LE, Lee JE, et al. Genome-wide association study identifies novel loci predisposing to cutaneous melanoma. *Hum Mol Genet*. 2011;20(24):5012–5023.

21. Williams PF, Olsen CM, Hayward NK, Whiteman DC. Melanocortin 1 receptor and risk of cutaneous melanoma: a meta-analysis and estimates of population burden. *Int J Cancer*. 2011;129(7):1730–1740.
22. Garcia-Borrón JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell Res*. 2005;18(6):393–410.
23. Garcia-Borrón JC, Abdel-Malek Z, Jimenez-Cervantes C. MC1R, the cAMP pathway, and the response to solar UV: extending the horizon beyond pigmentation. *Pigment Cell Melanoma Res*. 2014;27(5):699–720.
24. Beaumont KA, Shekar SN, Newton RA, et al. Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. *Hum Mol Genet*. 2007;16(18):2249–2260.
25. Beaumont KA, Shekar SN, Cook AL, Duffy DL, Sturm RA. Red hair is the null phenotype of MC1R. *Hum Mutat*. 2008;29(8):E88–E94.
26. Doyle JR, Fortin JP, Beinborn M, Kopin AS. Selected melanocortin 1 receptor single-nucleotide polymorphisms differentially alter multiple signaling pathways. *J Pharmacol Exp Ther*. 2012;342(2):318–326.
27. Duffy DL, Box NF, Chen W, et al. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Hum Mol Genet*. 2004;13(4):447–461.
28. Whiteman DC, Green AC. A risk prediction tool for melanoma? *Cancer Epidemiol Biomarkers Prev*. 2005;14(4):761–763.
29. Stefanaki I, Panagiotou OA, Kodala E, et al. Replication and predictive value of SNPs associated with melanoma and pigmentation traits in a southern European case-control study. *PLoS One*. 2013;8(2):e55712.
30. Penn LA, Qian M, Zhang E, et al. Development of a melanoma risk prediction model incorporating MC1R genotype and indoor tanning exposure: impact of mole phenotype on model performance. *PLoS One*. 2014;9(7):e101507.
31. Dwyer T, Stankovich JM, Blizzard L, et al. Does the addition of information on genotype improve prediction of the risk of melanoma and nonmelanoma skin cancer beyond that obtained from skin phenotype? *Am J Epidemiol*. 2004;159(9):826–833.
32. Davies JR, Randerson-Moor J, Kukalich K, et al. Inherited variants in the MC1R gene and survival from cutaneous melanoma: a BioGenoMEL study. *Pigment Cell Melanoma Res*. 2012;25(3):384–394.
33. Guida M, Strippoli S, Ferretta A, et al. Detrimental effects of melanocortin-1 receptor (MC1R) variants on the clinical outcomes of BRAF V600 metastatic melanoma patients treated with BRAF inhibitors. *Pigment Cell Melanoma Res*. 2016;29(6):679–687.
34. Landi MT, Kanetsky PA, Tsang S, et al. MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. *J Natl Cancer Inst*. 2005;97(13):998–1007.
35. Guida S, Bartolomeo N, Zanna PT, et al. Sporadic melanoma in south-eastern Italy: the impact of melanocortin 1 receptor (MC1R) polymorphism analysis in low-risk people and report of three novel variants. *Arch Dermatol Res*. 2015;307(6):495–503.
36. Raimondi S, Gandini S, Fargnoli MC, et al. Melanocortin-1 receptor, skin cancer and phenotypic characteristics (M-SKIP) project: study design and methods for pooling results of genetic epidemiological studies. *BMC Med Res Methodol*. 2012;12:116.
37. Bergman W, Gruis NA. Management of melanoma families. *Cancers (Basel)*. 2010;2(2):549–566.
38. de Snoo F, Gruis N. Familial melanoma [webpage on the Internet]. 2005. Available from: <http://atlasgeneticsoncology.org/Kprones/FamilialMelanomID10088.html>. Accessed March 10, 2018.
39. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. *J Invest Dermatol*. 2001;117(2):294–300.
40. Bishop DT, Demenais F, Iles MM, et al. Genome-wide association study identifies three loci associated with melanoma risk. *Nat Genet*. 2009;41(8):920–925.
41. Menin C, Vecchiato A, Scaini MC, et al. Contribution of susceptibility gene variants to melanoma risk in families from the Veneto region of Italy. *Pigment Cell Melanoma Res*. 2011;24(4):728–730.
42. Ghiorzo P, Bonelli L, Pastorino L, et al. MC1R variation and melanoma risk in relation to host/clinical and environmental factors in CDKN2A positive and negative melanoma patients. *Exp Dermatol*. 2012;21(9):718–720.
43. Pastorino L, Cusano R, Bruno W, et al. Novel MC1R variants in Ligurian melanoma patients and controls. *Hum Mutat*. 2004;24(1):103.
44. Tagliabue E, Gandini S, Garcia-Borrón JC, et al. Association of melanocortin-1 receptor variants with pigimentary traits in humans: a pooled analysis from the M-SKIP project. *J Invest Dermatol*. 2016;136(9):1914–1917.
45. Vanderweele TJ, Vansteelandt S. Odds ratios for mediation analysis for a dichotomous outcome. *Am J Epidemiol*. 2010;172(12):1339–1348.
46. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods*. 2013;18(2):137–150.
47. Horrell EM, Boulanger MC, D'Orazio JA. Melanocortin 1 receptor: structure, function, and regulation. *Front Genet*. 2016;7:95.
48. April CS, Barsh GS. Distinct pigimentary and melanocortin 1 receptor-dependent components of cutaneous defense against ultraviolet radiation. *PLoS Genet*. 2007;3(1):e9.
49. Hauser JE, Kadekaro AL, Kavanagh RJ, et al. Melanin content and MC1R function independently affect UVR-induced DNA damage in cultured human melanocytes. *Pigment Cell Res*. 2006;19(4):303–314.
50. Kadekaro AL, Chen J, Yang J, et al. Alpha-melanocyte-stimulating hormone suppresses oxidative stress through a p53-mediated signaling pathway in human melanocytes. *Mol Cancer Res*. 2012;10(6):778–786.
51. Wong SS, Ainger SA, Leonard JH, Sturm RA. MC1R variant allele effects on UVR-induced phosphorylation of p38, p53, and DDB2 repair protein responses in melanocytic cells in culture. *J Invest Dermatol*. 2012;132(5):1452–1461.
52. Mitra D, Luo X, Morgan A, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature*. 2012;491(7424):449–453.
53. Robles-Espinoza CD, Roberts ND, Chen S, et al. Germline MC1R status influences somatic mutation burden in melanoma. *Nat Commun*. 2016;7:12064.
54. Johansson PA, Pritchard AL, Patch AM, et al. Mutation load in melanoma is affected by MC1R genotype. *Pigment Cell Melanoma Res*. 2017;30(2):255–258.
55. Fargnoli MC, Pike K, Pfeiffer RM, et al. MC1R variants increase risk of melanomas harboring BRAF mutations. *J Invest Dermatol*. 2008;128(10):2485–2490.
56. Landi MT, Bauer J, Pfeiffer RM, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. *Science*. 2006;313(5786):521–522.
57. Nagore E, Reyes-Garcia D, Heidenreich B, Garcia-Casado Z, Requena C, Kumar R. TERT promoter mutations associate with MC1R variants in melanoma patients. *Pigment Cell Melanoma Res*. 2017;30(2):273–275.
58. Little J, Wilson B, Carter R, et al. Multigene panels in prostate cancer risk assessment: a systematic review. *Genet Med*. 2016;18(6):535–544.
59. Mealiffe ME, Stokowski RP, Rhees BK, Prentice RL, Pettinger M, Hinds DA. Assessment of clinical validity of a breast cancer risk model combining genetic and clinical information. *J Natl Cancer Inst*. 2010;102(21):1618–1627.
60. Lindström S, Schumacher FR, Cox D, et al. Common genetic variants in prostate cancer risk prediction: results from the NCI Breast and Prostate Cancer Cohort Consortium (BPC3). *Cancer Epidemiol Biomarkers Prev*. 2012;21(3):437–444.
61. Macinnis RJ, Antoniou AC, Eeles RA, et al. A risk prediction algorithm based on family history and common genetic variants: application to prostate cancer with potential clinical impact. *Genet Epidemiol*. 2011;35(6):549–556.
62. Chatterjee N, Park JH, Caporaso N, Gail MH. Predicting the future of genetic risk prediction. *Cancer Epidemiol Biomarkers Prev*. 2011;20(1):3–8.
63. Pearson TA, Manolio TA. How to interpret a genome-wide association study. *JAMA*. 2008;299(11):1335–1344.
64. Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomics research. *Nature*. 2003;422(6934):835–847.

65. Hernan MA, Clayton D, Keiding N. The Simpson's paradox unraveled. *Int J Epidemiol*. 2011;40(3):780–785.
66. Cargill J, Lucas RM, Gies P, et al. Validation of brief questionnaire measures of sun exposure and skin pigmentation against detailed and objective measures including vitamin D status. *Photochem Photobiol*. 2013;89(1):219–226.
67. Garcia-Borrón JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell Res*. 2005;18(6):393–410.
68. Duffy DL, Box NF, Chen W, et al. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Hum Mol Genet*. 2004;13(4):447–461.
69. Box NF, Wyeth JR, O'Gorman LE, Martin NG, Sturm RA. Characterization of melanocyte stimulating hormone receptor variant alleles in twins with red hair. *Hum Mol Genet*. 1997;6(11):1891–1897.
70. Sturm RA, Duffy DL, Box NF, et al. Genetic association and cellular function of MC1R variant alleles in human pigmentation. *Ann NY Acad Sci*. 2003;994:348–358.

Cancer Management and Research

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient. The manuscript management system is completely online and includes

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>

a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress