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MC1R variants and cutaneous melanoma risk according to histological type, body site, and Breslow thickness: a pooled analysis from the M-SKIP project

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Disclosure statement of potential conflicts of interest
None declared

Ethics approval and consent to participate
Each original case-control study contributing to the M-SKIP project was separately approved by an Ethics Committee and/or all study participants provided a written consent to participate in the study (in accordance with the laws and regulations in force in the country where the study was conducted). The M-SKIP project was based on the pooling of anonymized, individual-level data collected within the above case-control studies, and as such, it did not require further approval from an Ethics Committee.

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Abstract

Background: Little is known on whether melanocortin 1 receptor (*MC1R*) associated cutaneous melanoma (CM) risk varies depending on histological subtype and body site, and whether tumour thickness at diagnosis (the most important prognostic factor for CM patients) differs between *MC1R* variant carriers and wild-type individuals.

Objective: We studied the association between *MC1R* variants and CM risk by histological subtype, body site, and Breslow thickness, using the database of the M-SKIP project.

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Methods: We pooled individual data from fifteen case-control studies conducted during 2005–2015 in Europe and the USA. Study-specific, multi-adjusted odds ratios were pooled into summary odds ratios (SOR) and 95% confidence intervals (CI) using random-effects models.

Results: 6891 CM cases and 5555 controls were included. CM risk was increased among *MC1R* variant carriers vs. wild-type individuals. The increase in risk was comparable across histological subtypes (SOR for any variant vs. wild-type ranged between 1.57 and 1.70, always statistical significant) except acral lentiginous melanoma, for which no association emerged; and slightly greater on chronically (1.74, 95% CI 1.47–2.07) than intermittently (1.55, 95% CI 1.34–1.78) sun-exposed skin. CM risk was greater for those carrying 'R' vs. 'r' variants; correlated with the number of variants; and was more evident among individuals not showing the red hair colour phenotype. Breslow thickness was not associated with *MC1R* status.

Conclusion: *MC1R* variants were associated with an increased risk of CM of any histological subtype (except ALM) and occurring on both chronically and intermittently sun-exposed skin.

Keywords

Melanocortin 1 receptor; cutaneous melanoma; body site; histological subtype; Breslow thickness; pooled analysis

Introduction

Cutaneous melanoma (CM) has traditionally been classified based on its histological features [1], with the majority of CM falling into four subtypes: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), and acral lentiginous melanoma (ALM). This classification has been criticized due to its limited prognostic significance [1], but it retains importance as the differences among subtypes encompass several clinical and epidemiological characteristics, including the preferential body site of occurrence, association with patterns of sunlight exposure, frequency of somatic mutations, tumour thickness, age and geographical distribution, and trends in incidence rates [1–5].

SSM typically occurs on intermittently sun-exposed sites, while LMM is associated with chronic exposure to sunlight and usually occurs in the head and neck region at an older age than SSM (median 8^{th} vs. 5^{th} decade, respectively). NM is less clearly associated with patterns of sunlight exposure, can occur at any anatomical location, and is usually thicker than the other subtypes. Incidence rates increased steadily over the past decades for SSM and LMM but remained stable for NM. ALM is the most common subtype among dark-skinned populations and is typically found on palms, soles and under/around the nails [1,6–7]. CM subtypes also differ in the frequency of somatic mutations in genes known to play a role in melanomagenesis [1,3,8–10].

Ultraviolet (UV) light exposure is the most important environmental risk factor for CM [11–12]. CM risk is associated with other phenotypic characteristics such as the skin, eye and hair colour, the number of common and atypical naevi, and the skin phototype [13–14]. Research has also identified several melanoma susceptibility genes [15]: among these, one of the most studied is the gene encoding the melanocortin 1 receptor (MC1R). This is a G

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protein-coupled receptor mainly expressed on melanocytes that plays a key role in skin pigmentation [16]. MC1R signalling leads to the production and storage of the dark (black/brown), highly photoprotective pigment eumelanin in the melanosomes and their transfer to keratinocytes, thus increasing the ratio of eumelanin to pheomelanin, a red/yellow pigment less efficient at protecting the skin from UV-induced damage [16].

MC1R is a highly polymorphic gene with more than 100 non-synonymous variant alleles identified to date [17], some of which are highly prevalent, albeit with considerable geographical variability [18–21]. Carriers of certain *MC1R* variant alleles show characteristic phenotypic traits (e.g. red hair, pale skin, and freckles) and an increased sensitivity to sunlight [22], and are at greater CM risk compared to wild-type individuals [23–24]. Much less is known on whether *MC1R*-associated CM risk varies depending on histological subtype and body site, and whether tumour thickness at diagnosis (the most important CM prognostic factor [25]) differs between *MC1R* variant carriers and wild-type individuals. While an association between *MC1R* variants and tumour site, histology or thickness was reported by some authors [26–29], others did not detect any difference [30–31].

Here, we aimed to evaluate the association between *MC1R* gene variants and CM risk according to the tumour histological type, body site, and Breslow thickness, through a pooled analysis of individual data from the large, multicenter case-control study M-SKIP (Melanocortin-1-receptor gene, SKIn cancer and Phenotypic characteristics).

Methods

The M-SKIP project

Data for the present analyses were gathered through the M-SKIP project, which has been described previously [32]. Briefly, 38 investigators (out of 49 that were contacted) consented to provide data from published and unpublished epidemiological studies focusing on *MC1R* variants, sporadic CM, non-melanoma skin cancer, and phenotypic characteristics associated with skin cancer. Case-control studies contributing to the M-SKIP database did not differ from those conducted from non-participating investigators in terms of sex and age distribution, source of controls, methods for assessment of phenotypic characteristics, and laboratory methods [24]. For the present analysis, we selected data from case-control studies in which the *MC1R* gene was sequenced, information was available on histological subtype and/or body site and/or Breslow thickness, and a control group was available for comparison.

MC1R variants and study outcomes

We aimed to determine CM risk (by histological subtype, body site, and Breslow thickness) among carriers of *MC1R* variants vs. wild-type individuals. Among the nine most common *MC1R* variants, six (D84E, D294H, I155T, R142H, R151C, and R160W) were previously shown to be strongly associated with the red hair phenotype and are usually labelled as ‘R’ variants, and three (R163Q, V60L, and V92M) are associated with red hair to a lesser degree and are labelled as ‘r’ variants [33–34]. In fact, the I155T variant is labelled as a ‘r’ by some

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authors (e.g. in [21], but for consistency with previous investigations conducted in the M-SKIP database, we preferred to maintain the above classification. For rarer *MC1R* variants, we defined R and r variants according to their likely pathogenicity using bioinformatics analysis [31]. Several models were fitted in which the "exposure" (or "exposure level") was defined as carrying: (1) at least one *MC1R* variant (any type); (2) only one or at least two variants (any type); (3) only one or at least two 'r' variants; (4) only one or at least two 'R' variants; and (5) each one of the nine most common variants. Wild-type individuals were considered as "unexposed" in all of the models that were fitted.

Melanomas of the following histological subtypes were separately considered as study outcomes: SSM, NM, LMM (for brevity, we will use this acronym to encompass both lentigo malignant melanoma and its in situ form, lentigo maligna), ALM, and other subtypes (e.g. spitzoid, nevoid, epithelioid, desmoplastic, and others not specified); melanomas with mixed histology were not considered. In terms of body site, we separately evaluated CM arising on intermittently (trunk and lower limbs) or chronically (upper limbs and head and neck) sunlight-exposed skin. Melanomas occurring on mostly sunlight-unexposed skin (i.e. pubis, groin, armpit, buttock, vulva, palm, sole, subungual, pelvis, perianal, anal, penis and vagina) were not considered because they were not reported for all the studies and their number was very small (73 cases from six studies). Finally, we performed analyses stratified by thickness using the categorization in four classes (≤1, >1–2, >2–4, and >4 mm) from the American Joint Committee on Cancer melanoma staging system, 8th edition [35].

Statistical analysis

Statistical analyses were conducted according to a two-stage procedure as previously described [32]. First, multinomial logistic regression models were fitted to data from each study, in order to obtain study-specific odds ratio (OR) and corresponding 95% confidence intervals (CI). When available, the following covariates were included in each study-specific model: age, sex, family history of melanoma, total body count of common melanocytic naevi, presence of atypical naevi, number of lifetime and childhood sunburns, and (for models focusing on histological subtypes and Breslow thickness) intermittent and chronic exposure to sunlight. Second, study-specific ORs were pooled into a summary odds ratio (SOR) using random-effects models [36]. No SOR was calculated when there were fewer than 10 cases. We then performed meta-regression analyses comparing SORs for each subtype/body site/Breslow thickness category, using as reference SSM for subtype, chronically exposed skin for body site, and <1 mm for Breslow thickness. In line with our previous analyses [37], all analyses were repeated after stratification for the red hair colour (RHC) phenotype (defined as the presence of any of red hair, freckles, and skin type I/II). We assumed dominant inheritance of *MC1R* variants in all analyses, since statistical models assuming alternative modes of inheritance showed a worse fit in previous analyses of the M-SKIP database [24].

We calculated the I^2 index to assess the percentage of total variation across studies that is attributable to true heterogeneity rather than chance [38]. When I^2 was ≥50%, which has been interpreted as indicating moderate or greater heterogeneity [39], we performed meta-regression and subgroup analysis to assess whether study-specific OR varied by publication

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year (as a continuous variable), geographic location (Southern Europe vs. Northern Europe and USA), or source of controls (population vs. hospital). Finally, the presence of small study effects was graphically assessed by funnel plots and formally tested using the Egger's test [40].

All analyses were conducted using SAS (version 9.4, Cary, NC, USA) and STATA (version 11.2, Lakeway, TX, USA). All tests were two-sided and p-values of less than 0.05 were considered as statistically significant.

Results

We pooled data from fifteen independent case-control studies (Table 1) conducted between 2005 and 2015 in Europe and the USA, and included in the analysis 6,891 melanoma cases (mean age 52 years, 46% males) and 5,555 controls (mean age 48 years, 51% males). Melanoma cases were compared to healthy controls in ten studies, and to hospital controls in five studies. Tumour data (histological subtype, body site, and Breslow thickness) were collected in all case-control studies (although not for all CM patients in all studies), except the study carried out by Scherer et al. in Germany, which had information on histological subtype only. Information on age, sex and family history of melanoma was available for all participants in all studies; information on sunlight exposure, sunburns, common and atypical naevi was available in six, ten, six and six papers, respectively (Table 1). Most included CM cases occurred on intermittently sun-exposed skin (68.6%) and belonged to the superficial spreading histological subtype (62.2%) (Supplementary Table 1). The median Breslow thickness was higher for NM (2.9 mm) and ALM (2.0 mm) compared to SSM (0.8 mm) and LMM (0.5 mm) (age- and sex-adjusted $p < 0.0001$), and for melanoma occurring on chronically vs. intermittently sun-exposed skin (1.1 vs. 1.0 mm, respectively; age- and sex-adjusted $p = 0.016$).

MC1R and risk of cutaneous melanoma according to histological subtype

Carriers of at least one *MC1R* variant had a 57-to-70% increased risk to develop a CM of any histological subtype except ALM (SOR 0.96, 95%CI 0.70–1.31) (Table 2 and Supplementary Figure 1). For all histological subtypes except ALM, the increase in risk ranged between 33% and 51% for carriers of one variant, and above two-fold for individuals carrying two or more variants. In addition, *MC1R* 'r' variants conveyed a lower melanoma risk compared to 'R' variants. The heterogeneity between studies was generally low (I^2<50%), except for the association of two *MC1R* variants with SSM (any type and 'R') and LMM ('R') (Table 2). Meta-regression models detected an effect of publication year on the association of two or more *MC1R* variants (vs. wild type) with SSM ($\beta = 0.86$, p-value 0.04), while there was no evidence of small study effects.

No single *MC1R* variant was significantly associated with an increased ALM risk (Supplementary Table 2). For all the other histological subtypes, each single *MC1R* 'R' variant conveyed a stronger risk than each single 'r' variant, with the only exception being the association between the D294H 'R' variant and LMM risk, as the SOR was the second lowest and did not achieve statistical significance.

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The results of the analysis stratified by RHC phenotype are shown in Figure 1. The risk of CM of any histological subtype did not differ between *MC1R* variant carriers vs. wild-type individuals showing the RHC phenotype. Instead, among non-RHC individuals, *MC1R* gene variant carriers had a significantly increased risk of SSM (SOR 1.90, 95%CI 1.24–1.93) and NM (SOR 1.74, 95%CI 1.03–2.94), and a non-significant increase in LMM and ALM risk (by 41% and 98%, respectively), compared to wild type individuals. Of note, while LMM risk was associated with MC1R status in the whole study population (Table 2), this was not observed in RHC phenotype-stratified analyses (Figure 1), most likely because of the reduction in statistical power due to the stratification.

MC1R and risk of cutaneous melanoma according to body site

Individuals carrying at least one *MC1R* variant had a significantly increased CM risk on both chronically (SOR 1.74, 95% CI 1.47–2.07) and intermittently (SOR 1.55, 95% CI 1.34–1.78) sun-exposed skin (Table 3 and Supplementary Figure 2). The association emerged among patients carrying ‘r’ or ‘R’ *MC1R* variants, although the association was generally stronger for ‘R’ variants, and the rise in melanoma risk correlated with the number of *MC1R* variants, regardless of the variant type (‘r’ or ‘R’). In addition, the increase in risk was somewhat, albeit not significantly, stronger for melanomas occurring on chronically vs. intermittently sun-exposed skin. The I^2 was nearly always <50%, except for the SOR calculated among carriers of two or more ‘R’ variants (I^2 was 52.7% and 61.5% for melanoma occurring on chronically and intermittently sun-exposed skin, respectively); however, no study characteristics were associated with study-specific OR in meta-regression models.

The above findings were confirmed when evaluating CM risk associated with each single *MC1R* variant (Supplementary Table 3). Carriers of each *MC1R* variant were at significantly increased risk of developing melanoma on both chronically sun-exposed skin (with no exceptions) and intermittently sun-exposed skin (except among carriers of the D84E ‘R’ or the R163Q ‘r’ variants), with the association being generally stronger for chronically sun-exposed skin. In addition, each single *MC1R* ‘R’ variant conveyed a greater CM risk than each single ‘r’ variant, on both intermittently and chronically sun-exposed skin. Between-study heterogeneity was low and not accounted for by any variable among those tested in meta-regression and subgroup analysis when I^2 was above 50% (i.e. for the R151C variant and, limited to melanoma on chronically sun-exposed skin, the I155T variant).

Among RHC individuals, those carrying at least one *MC1R* ‘R’ variant were at increased CM risk on both chronically and intermittently sun-exposed skin (the association being stronger for chronically sun-exposed skin), while no association emerged for ‘r’ variants. Instead among non-RHC individuals, both ‘R’ and ‘r’ *MC1R* variants were associated with an increased CM risk, with no appreciable differences in magnitude by body site.

MC1R and risk of cutaneous melanoma according to Breslow thickness

Melanoma risk associated with *MC1R* variant status did not vary across categories of Breslow thickness, as variant carriers had an increased melanoma risk of about 50% irrespective of Breslow thickness at diagnosis, with generally low between-studies

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heterogeneity (Table 4 and Supplementary Figure 3). As above, the increase in melanoma risk correlated with the number of variants, and was stronger for carriers of 'R' than 'r' variants; however, SORs did not differ between melanoma of different thickness. The same pattern emerged when focusing on the association between each single *MC1R* variant and the risk of melanoma stratified by Breslow thickness (Supplementary Table 4). Results did not differ in analyses stratified by RHC phenotype (results not shown).

Discussion

We conducted a pooled analysis of data from the M-SKIP study (6,891 CM cases and 5,555 controls from fifteen independent studies), and found that *MC1R* gene variants conferred an increase in CM risk that was comparable across histological subtypes (except ALM, for which no association was seen) and body sites. The association with CM risk emerged for carriers of both 'R' and (with few exceptions) 'r' gene variants, although generally stronger for 'R' variants. Moreover, there was evidence of a dose-response effect of *MC1R* gene variant number on CM risk. The increase in CM risk was more evident when comparing *MC1R* variant carriers vs. wild-type non-RHC individuals, and weaker (and limited to 'R' variants) when restricting the analysis to RHC individuals. Finally, Breslow thickness did not differ between *MC1R* gene variant carriers and wild-type individuals. The heterogeneity among studies was generally moderate and not accounted for by differences in study characteristics, except for stronger SSM risk in more recent studies among carriers of two or more *MC1R* gene variants.

MC1R signalling is involved in several cellular pathways in melanocytes, which can help explain why CM risk among variant carriers is increased for most histological subtypes and skin sites. Activation of the cAMP (3'−5'-cyclic adenosine monophosphate) signalling cascade is the main mechanism by which MC1R regulates skin pigmentation [17,41]. *MC1R* 'R' alleles yield receptors with severely decreased ability to activate the cAMP cascade, which is instead only moderately impaired in 'r' receptors: this may account for the much stronger association of 'R' alleles with the RHC phenotype [17]. Eumelanin deficiency is probably the most important phenotype-mediated mechanism of melanomagenesis among *MC1R* variant carriers, along with the ability of pheomelanin to act as a photosensitizing agent [42]. However, the physiological role of *MC1R* signalling in melanocytes extends to several other cellular pathways [43], which may also be impaired among variant carriers and contribute to CM risk. In particular, some of these pathways are independent of the phenotype (because not mediated by the cAMP cascade), and may therefore account for the "direct" effect of *MC1R* variants on CM risk [37].

The strength of the association between *MC1R* variants and CM risk was comparable for SSM, NM, e LMM, despite their diversity in terms of association with patterns of exposure to sunlight and body site distribution. With few exceptions, the above held true when separately considering each single *MC1R* variant; in particular, we did not observe the previously reported association between the R163Q variant and LMM risk [28]. The increase in SSM risk over time among carriers of 2+ 'R' variants is difficult to interpret, all the more so considering that no similar trend was observed for LMM in the same subset of participants. ALM risk was instead not increased among *MC1R* variant carriers, and was

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even inversely associated (albeit not significantly) with *MC1R* variants among RHC individuals. ALM is the most common histological subtype in dark-complexioned individuals, seems not to be related to sun exposure [44–45], and is characterized by a distinct range of genetic aberrations [46–47]. Thus, our findings corroborate literature data that highlights the diversity of ALM from the other CM histological subtypes. However, given the relatively small number of ALM cases in the M-SKIP database and the fact that the mechanisms underlying the development of this subtype are still unclear, our findings on ALM should be considered with caution.

MC1R variant carriers were at increased CM risk on both chronically and intermittently sun-exposed skin. According to Whiteman's divergent pathway model for melanoma development [48], CM on occasionally sun-exposed skin are associated with higher naevi count, intermittent UV exposure, and a history of sunburns. Instead, CM on usually sun-exposed body sites and the LMM histological subtype are associated with signs of chronic exposure to sunlight (e.g. actinic lesions). However, no evidence of an association between chronic sunlight exposure and CM risk at any site emerged in previous meta-analyses [49]. In finding an association between *MC1R* variants and CM risk generally, our study suggests that the association between chronic sun exposure and CM risk might be limited to individuals carrying germline variants of the *MC1R* gene. In fact, the association with *MC1R* variants was even stronger for CM on chronically vs. intermittently sun-exposed skin in our study. A possible explanation is that subjects with high skin sensitivity may more often adopt sun-avoidance behaviours and limit sunlight exposure of the trunk and lower limbs, while the head and neck are more difficult to shield from UV radiation.

The findings of the analyses stratified by phenotype are in line with our previous reports [37], and suggest that the "direct" (i.e. not phenotype-mediated) effect of *MC1R* variants on CM risk is of limited importance among those who are already at high CM risk because of their RHC phenotype. Finally, the lack of an association between *MC1R* gene variants and Breslow thickness is consistent with previous reports [27,29–31] and can be explained by the comparable increase in the risk of thicker (e.g. NM) and thinner (e.g. SSM) subtypes.

The major strength of our analysis is its large sample size, which allowed investigating with unprecedented statistical power the association between *MC1R* gene variants and the risk of histological subtype-, body site-, and Breslow thickness-specific CM. The heterogeneity between study-specific risk estimates was generally low, which strengthens the reliability of our results. Our study has also some limitations. Information on potential confounders was not available in all studies, which may have introduced some bias in study-specific risk estimates. No information was available on genes having an effect on skin phenotype and, therefore, potentially able to interact with *MC1R* gene variants in determining CM risk (e.g. *OCA2*, *ASIP*, and *TYRP1*). All case-control studies included in the M-SKIP were conducted in Europe or the USA, which curbed the statistical power of our study where it focused on the ALM histological subtype. Finally, we performed a relatively high number of statistical tests in univariate analysis, which may raise a problem of multiple testing. We believe that our approach of simply describing what tests of significance have been performed, as advised by Perneger [50], is appropriate, given the explorative (rather than

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confirmative) nature of the study and the existence of a biological rationale for the associations that were investigated.

In conclusion, we found that the effect of *MC1R* gene variants on CM risk extends to most histological subtypes and body sites, but does not affect the Breslow thickness at diagnosis. Future studies should aim to achieve a better understanding of how *MC1R* gene variants interact with other known genetic and environmental risk factors in determining CM risk among these individuals, and identify which, among the multiple biological pathways regulated by MC1R in melanocytes, contribute most to raising CM risk among carriers of each specific gene variant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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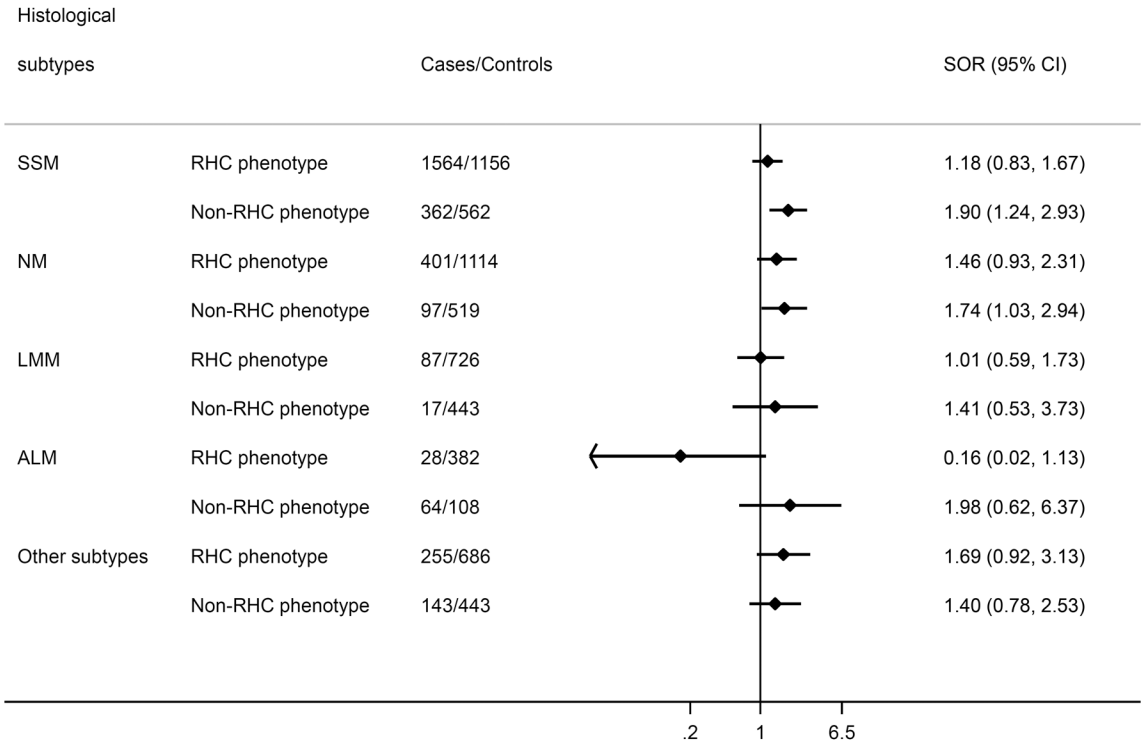

Figure 1.

Summary odds ratio (SOR) and 95% confidence intervals (CI) for the association between any *MC1R* variants and cutaneous melanoma according to histological subtype stratified by presence of the red hair colour (RHC) phenotype.

SSM: superficial spreading melanoma

NM: nodular melanoma

LMM: lentigo maligna melanoma

ALM: acral lentiginous melanoma

Study-specific odds ratios were adjusted for the following covariates (when available): age, sex, family history of melanoma, total body count of common naevi, presence of atypical naevi, number of lifetime and childhood sunburns, and reported intermittent and chronic exposure to sunlight.

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Table 1.

Characteristics of the fifteen case-control studies included in the pooled analysis. The M-SKIP (Melanocortin-1 receptor gene, SKIn cancer and Phenotypic characteristics) project.

Principal investigator, year	Country	Controls type[(a)]	No. cases	No. controls	Mean age (SD)		% of males		Available confounders[(b)]
					Cases	Controls	Cases	Controls	
Landi, 2005	Italy	healthy	136	171	49 (15)	46 (13)	48	49	sun exposure, sunburns
Stratigos, 2006	Greece	hospital	108	155	52 (15)	44 (15)	54	54	sun exposure, sunburns, common and atypical naevi
Fargnoli, 2006	Italy	hospital	136	163	49 (14)	49 (14)	50	50	sun exposure, sunburns, common and atypical naevi
Fernandez, 2007	Spain	healthy	100	188	51 (15)	53 (14)	39	46	sunburns, common naevi
Bishop, 2009	UK	hospital	1,529	496	54 (14)	56 (13)	43	41	sunburns
Casula, 2009	Italy	healthy	156	75	50 (15)	61 (15)	49	27	-
Hoiom, 2009	Sweden	healthy	524	477	55 (18)	42 (12)	49	64	-
Scherer (S), 2009[(c)]	Spain	healthy	1,323	558	54 (17)	37 (12)	45	62	-
Scherer (G), 2009[(c)]	Germany	healthy	512	1,064	58 (15)	54 (12)	56	56	-
Kanetsky, 2010	USA	healthy	709	325	49 (14)	48 (13)	49	43	sun exposure, sunburns, atypical naevi
Menin, 2011	Italy	healthy	103	170	45 (14)	41 (11)	42	65	sunburns, common and atypical naevi
Ghiorzo, 2012	Italy	healthy	197	507	52 (16)	51 (17)	49	47	sunburns
Puig, 2013	Spain	hospital	424	331	54 (17)	42 (11)	46	53	atypical naevi
Penn, 2014	USA	healthy	884	769	47 (9)	46 (9)	40	42	sun exposure, sunburns, common naevi
Guida, 2015	Italy	hospital	50	106	55 (15)	51 (18)	56	52	sun exposure, sunburns, common and atypical naevi
Total			**6,891**	**5,555**	**52 (15)**	**48 (14)**	**46**	**51**	

[(a)] Healthy controls may include population controls, friends or partners of cases, outpatients or hospital personnel.

[(b)] Age, sex, and family history of melanoma were available in all 15 studies. Confounders with more than 20% of missing data were not listed. Sun exposure included separate information on chronic and intermittent sun exposure.

[(c)] S: Spanish participants; G: German participants.

SSM: superficial spreading melanoma; NM: nodular melanoma; LMM: lentigo maligna melanoma; ALM: acral lentiginous melanoma.

SD: standard deviation.

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Table 2.

Summary odds ratio (SOR) and 95% confidence intervals (95%CI) for the association between combined MC1R variants and cutaneous melanoma according to histological subtype.

MC1R variant	Histological subtype[(a)]	No. studies	No. cases	No. controls	SOR	95% CI	I^2	p-value[(b)]
Wild-type	SSM	15	957	1803				
	NM	15	271	1803				
	LMM	12	95	1627	reference			
	ALM	11	97	1383				
	Other subtypes	6	154	1242				
Any variant	SSM	15	3007	3270	1.57	1.33–1.84	39.9%	reference
	NM	15	853	3270	1.70	1.42–2.02	0.0%	0.57
	LMM	12	248	2999	1.65	1.24–2.19	0.0%	0.83
	ALM	11	134	2457	0.96	0.70–1.31	0.0%	**0.004**
	Other subtypes	6	552	2451	1.60	1.30–1.97	0.0%	0.97
1 variant	SSM	15	1702	2368	1.34	1.17–1.53	8.9%	reference
	NM	15	500	2368	1.51	1.25–1.83	0.0%	0.31
	LMM	12	149	2158	1.50	1.10–2.05	0.0%	0.52
	ALM	11	107	1755	1.02	0.72–1.46	51.0%	0.15
	Other subtypes	6	321	1743	1.33	1.07–1.66	0.0%	0.95
2+ variants	SSM	15	1305	902	2.16	1.69–2.76	54.4%	reference
	NM	15	353	902	2.29	1.69–3.11	32.1%	0.92
	LMM	9	98	815	2.13	1.50–3.02	0.0%	0.94
	ALM	7	33	587	0.81	0.51–1.27	0.0%	**0.001**
	Other subtypes	5	231	676	2.28	1.78–2.93	0.0%	0.85
1 ‘r’ variant	SSM	15	915	1503	1.15	0.96–1.39	35.4%	reference
	NM	14	227	1497	1.12	0.90–1.39	0.0%	0.83
	LMM	10	93	1307	1.47	1.04–2.08	0.0%	0.21
	ALM	9	69	935	1.05	0.72–1.52	0.0%	0.63
	Other subtypes	6	171	1088	1.25	0.98–1.61	0.0%	0.59
2+ ‘r’ variants	SSM	13	263	313	1.30	0.94–1.80	39.5%	reference
	NM	12	74	307	1.44	1.04–2.02	0.0%	0.59
	LMM	6	21	248	1.44	0.82–2.54	0.0%	0.73
	ALM	4	9	103	-	-	-	0.63
	Other subtypes	5	47	237	1.40	0.95–2.07	0.0%	0.77
1 ‘R’ variant	SSM	15	1458	1282	1.92	1.61–2.28	28.0%	reference
	NM	15	458	1282	2.34	1.91–2.86	0.0%	0.11
	LMM	10	99	1139	2.04	1.56–2.67	0.0%	0.69
	ALM	10	57	851	1.04	0.70–1.54	0.0%	**0.006**
	Other subtypes	6	263	1004	1.77	1.40–2.24	0.0%	0.59
2+ ‘R’ variants	SSM	14	355	144	3.43	2.08–5.64	62.9%	reference
	NM	12	87	139	4.38	3.00–6.39	0.0%	0.62

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MC1R variant	Histological subtype[(a)]	No. studies	No. cases	No. controls	SOR	95% CI	I^2	p-value[(b)]
	LMM	5	27	106	6.07	2.50–14.7	52.7%	0.24
	ALM	2	3	38	-	-	-	0.57
	Other subtypes	5	71	97	4.71	3.13–7.08	0.0%	0.62

(a) Melanoma cases with mixed histology were excluded from this analysis.

(b) Meta-regression p-value for the difference of SOR among subtypes, considering SSM as reference category.

Study-specific odds ratios were adjusted for the following covariates (when available): age, sex, family history of melanoma, total body count of common naevi, presence of atypical naevi, number of lifetime and childhood sunburns, and intermittent and chronic exposure to sunlight.

MC1R 'R' variants are the following: D84E, D294H, I155T, R142H, R151C, and R160W, while 'r' variants are R163Q, V60L, and V92M.

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Table 3.

Summary odds ratio (SOR) and 95% confidence intervals (95%CI) for the association between combined MC1R variants and cutaneous melanoma according to body site.

MC1R variant	Body site	No. studies	No. cases	No. controls	SOR	95% CI	I^2	p-value[(a)]
Wild-type	Chronically exposed skin[(b)]	14	388	1452	reference			
	Intermittently exposed skin[(c)]	14	933	1452				
Any variant	Chronically exposed skin[(b)]	14	1345	2562	1.74	1.47–2.07	10.4%	reference
	Intermittently exposed skin[(c)]	14	2848	2562	1.55	1.34–1.78	19.6%	0.24
1 variant	Chronically exposed skin[(b)]	14	722	1869	1.40	1.18–1.65	0.0%	reference
	Intermittently exposed skin[(c)]	14	1668	1869	1.36	1.20–1.54	0.0%	0.82
2+ variants	Chronically exposed skin[(b)]	13	623	675	2.72	2.02–3.65	46.6%	reference
	Intermittently exposed skin[(c)]	14	1180	693	2.01	1.57–2.57	47.9%	0.16
1 'r' variant	Chronically exposed skin[(b)]	14	248	1190	1.26	1.02–1.54	7.7%	reference
	Intermittently exposed skin[(c)]	14	871	1190	1.17	0.98–1.40	17.9%	0.61
2+ 'r' variants	Chronically exposed skin[(b)]	12	122	236	1.82	1.15–2.90	45.2%	reference
	Intermittently exposed skin[(c)]	12	248	236	1.54	1.03–2.31	49.7%	0.65
1 'R' variant	Chronically exposed skin[(b)]	13	647	985	2.07	1.73–2.48	0.0%	reference
	Intermittently exposed skin[(c)]	14	1402	1007	1.89	1.59–2.26	23.2%	0.45
2+ 'R' variants	Chronically exposed skin[(b)]	11	180	98	5.14	2.96–8.94	52.7%	reference
	Intermittently exposed skin[(c)]	14	314	113	2.80	1.61–4.87	61.5%	0.16

[(a)]Comparison of SOR for chronically (reference) vs. intermittently exposed skin.

[(b)]Trunk and lower limbs.

[(c)]Head, neck and upper limbs.

Study-specific odds ratios were adjusted for the following covariates (when available): age, sex, family history of melanoma, total body count of common naevi, presence of atypical naevi, and number of lifetime and childhood sunburns.

MC1R 'R' variants are the following: D84E, D294H, I155T, R142H, R151C, and R160W, while 'r' variants are R163Q, V60L, and V92M.

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Table 4.

Summary odds ratio (SOR) and 95% confidence intervals (95%CI) for the association between combined *MC1R* variants and cutaneous melanoma according to Breslow thickness.

MC1R variant	Breslow thickness	No. studies	No. cases	No. controls	SOR	95% CI	I^2	p-value[(a)]	p-value for trend
Wild-type	≤ 1 mm	14	668	1392	reference				
	>1–2 mm	14	311	1392					
	>2–4 mm	14	219	1392					
	> 4 mm	12	143	1292					
Any variant	≤ 1 mm	14	2053	2562	1.51	1.30–1.76	17.9%	reference	
	>1–2 mm	14	1062	2562	1.64	1.33–2.03	20.9%	0.56	
	>2–4 mm	14	637	2562	1.47	1.12–1.91	23.8%	0.93	
	> 4 mm	12	351	2364	1.49	1.07–2.09	28.0%	0.84	0.74
1 variant	≤ 1 mm	14	1173	1869	1.28	1.12–1.47	0.0%	reference	
	>1–2 mm	14	595	1869	1.41	1.17–1.69	0.0%	0.44	
	>2–4 mm	14	382	1869	1.39	1.12–1.71	0.0%	054	
	> 4 mm	12	216	1709	1.36	1.04–1.77	0.0%	0.73	0.60
2+ variants	≤ 1 mm	14	880	693	2.10	1.62–2.73	46.6%	reference	
	>1–2 mm	13	467	675	2.54	1.80–3.59	46.5%	0.45	
	>2–4 mm	14	255	693	1.88	1.32–2.68	25.2%	0.71	
	> 4 mm	12	135	655	1.83	1.00–3.35	55.6%	0.75	0.52
1 ‘r’ variant	≤ 1 mm	14	631	1181	1.12	0.91–1.37	27.7%	reference	
	>1–2 mm	14	304	1181	1.18	0.90–1.53	21.9%	0.70	
	>2–4 mm	13	192	1150	1.15	0.89–1.50	5.8%	0.89	
	> 4 mm	11	120	1071	1.22	0.88–1.69	6.0%	0.69	0.77
2+ ‘r’ variants	≤ 1 mm	11	186	230	1.29	0.90–1.83	33.7%	reference	
	>1–2 mm	12	90	236	1.45	0.91–2.32	31.4%	0.84	
	>2–4 mm	11	55	228	1.25	0.83–1.86	0.0%	0.99	
	> 4 mm	9	36	199	2.05	1.27–3.31	0.0%	0.13	0.28
1 ‘R’ variant	≤ 1 mm	14	990	1007	1.84	1.49–2.27	34.8%	reference	
	>1–2 mm	14	535	1007	2.05	1.62–2.58	13.8%	0.52	
	>2–4 mm	14	315	1007	1.83	1.44–2.32	0.0%	0.96	
	> 4 mm	11	165	896	1.78	1.27–2.49	10.3%	0.80	0.78
2+ ‘R’ variants	≤ 1 mm	13	237	110	2.76	1.59–4.81	59.4%	reference	
	>1–2 mm	12	129	104	4.61	2.54–8.37	47.1%	0.24	
	>2–4 mm	9	69	95	3.68	1.79–7.57	43.2%	0.56	
	> 4 mm	7	28	92	4.20	1.45–12.21	52.6%	0.42	0.50

*(a)*Meta-regression p-value for the difference of SOR among Breslow thickness categories, considering melanomas ≤1 mm as reference category.

Study-specific odds ratios were adjusted for the following covariates (when available): age, sex, family history of melanoma, total body count of common naevi, presence of atypical naevi, number of lifetime and childhood sunburns, and intermittent and chronic exposure to sunlight.

MC1R ‘R’ variants are the following: D84E, D294H, I155T, R142H, R151C, and R160W, while ‘r’ variants are R163Q, V60L, and V92M.