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MC1R genotypes and risk of melanoma before age 40 years: a population-based case-control-family study

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Abstract

The contribution of melanocortin-1 receptor (*MC1R*) gene variants to the development of early-onset melanoma is unknown. Using an Australian population-based, case-control-family study, we sequenced *MC1R* for 565 cases with invasive cutaneous melanoma diagnosed between ages 18–39 years, 409 unrelated controls and 518 sibling controls. Variants were classified *a priori* into `R' variants (D84E, R142H, R151C, I155T, R160W, D294H) and `r' variants (all other nonsynonymous variants). We estimated odds ratios (OR) for melanoma using unconditional (unrelated controls) and conditional (sibling controls) logistic regression. The prevalence of having at least one R or r variant was 86% for cases, 73% for unrelated controls and 81% for sibling controls. R151C conferred the highest risk (per allele OR 2.57, 95% confidence interval 1.86–3.56 for the case-unrelated-control analysis and 1.70 (1.12–2.60) for the case-sibling-control analysis). When mutually adjusted, the ORs per R allele were 2.23 (1.77–2.80) and 2.06 (1.47–2.88), respectively from the two types of analysis, and the ORs per r allele were 1.69 (1.33–2.13) and 1.25 (0.88–1.79), respectively. The associations were stronger for men and those with none or few nevi or with high childhood sun exposure. Adjustment for phenotype, nevi and sun exposure attenuated the overall log OR for R variants by approximately 18%, but had lesser influence on r variant risk estimates. *MC1R* variants explained about 21% of the familial aggregation of melanoma. Some *MC1R* variants are important determinants of early-onset melanoma. The strength of association with melanoma differs according to the type and number of variants.

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Keywords

MC1R; melanoma; early-onset; phenotype; nevi; sun exposure

INTRODUCTION

The production of melanin in skin and hair melanocytes, and consequently the determination of skin and hair colour, is partly regulated by the melanocortin-1 receptor *(MC1R)* gene, which encodes the melanocyte-stimulating hormone receptor.[1] There are many common variants of *MC1R* associated with phenotypes such as red or blonde hair, fair skin, freckling, and skin sensitivity to ultraviolet (UV) light.[2] Several of these variants are associated with increased melanoma risk,[3–6] however not all of these associations have been attributed to phenotype[7–9] suggesting that these variants affect melanoma risk independently of phenotype. Carrying an *MC1R* variant may also negate the protection normally afforded by darker complexion.[4, 8, 9]

Although *MC1R* variants have been labelled `low risk' in terms of their influence on melanoma risk,[10] as a group they might account for a substantial proportion of disease in the population. This could be particularly relevant for early-onset melanoma because those who have melanoma diagnosed when young are likely to have a stronger underlying genetic susceptibility to the disease. In addition, the increased risk associated with carrying these variants could be greater for subgroups with specific phenotypes or UV exposures.

For young adults of European origin, melanoma is one of the more common cancers and is a leading cause of cancer death.[11–13] It is unknown to what extent *MC1R* variants contribute to the development of early-onset melanoma and if their associations differ according to phenotype and environmental factors. We addressed these questions using data from the Australian Melanoma Family Study, a multi-centre, population-based, case-control-family study of invasive cutaneous melanoma diagnosed between ages 18 and 39 years. Taking advantage of the family design, we conducted analyses using sibling as well as unrelated controls (population controls and spouse or friend controls).

MATERIAL AND METHODS

Subjects

The study design, recruitment, data collection and subject characteristics have been previously described in detail.[14] Briefly, at the time of recruitment, cases and controls were living in the greater urban areas of Brisbane (27.3°S, high ambient UV), Sydney (33.6°S, intermediate ambient UV) or Melbourne (37.5°S, lower ambient UV), the three largest urban populations in Australia comprising about 50% of the country's population.

Cases—Cases were identified from population-based state cancer registries and were eligible if diagnosed between 1st July 2000 and 31st December 2002 at ages 18–39 years with incident, histopathologically-confirmed, first-primary invasive cutaneous melanoma. A total of 629 cases were recruited; participation was 54% of those eligible and 76% of those contactable. Blood samples were requested from all participants, and were obtained from

597 (95%) cases. The median interval between diagnosis of melanoma and interview was 10.0 months (25^{th}–75^{th} centile: 6.8–14.1 months).

Controls—Population controls were aged between 18 and 39 years at the time of approach and had no history of invasive or *in situ* melanoma. They were selected from the electoral roll (registration to vote is compulsory for Australian citizens aged 18 years and over) and were frequency-matched to cases by city, age (within 5 years) and sex. A total of 240 population controls were recruited; participation was 23% of those apparently eligible and 42% of those contactable. A blood sample was obtained from 220 (92%) population controls.

Eligible spouse or friend controls were a spouse, partner, or friend nominated by a case as a potential control subject. They were eligible if they were at least 18 years of age and had no history of invasive or *in situ* melanoma; there were no other age, sex or residency restrictions. A spouse or friend was nominated as a potential control subject by 59% of cases. A total of 295 spouse or friend controls were recruited; participation was 80% of those nominated. A blood sample was obtained from 256 (87%) spouse or friend controls.

We sought to recruit all siblings of cases for whom the case permitted contact. Unaffected siblings of cases were considered as sibling controls for this analysis. At least one full sibling without any reported melanoma was identified for 560 (89%) cases (total 1276 siblings), and a blood sample was obtained from 624 (49%) sibling controls.

Questionnaire

A structured questionnaire administered by telephone interview included questions on past sun exposure, phenotype, residence history, demographic information, ancestry and cancer diagnoses.[14] Case and control participants reported their own and relatives' histories of melanoma and other cancers. Verification of reported cancers was sought from cancer registries, hospital and pathology records, treating clinicians, general practitioners and death certificates, when participant or next-ofkin (if participant deceased) consent had been obtained.[14] Measurement of childhood (up to and including 17 years of age) and lifetime sun exposure and ambient UV radiation exposure have been described in detail.[14] Participants also reported their skin, eye colour, natural hair colour at age 18 years, usual tanning and sunburn response to prolonged or repeated exposure of skin to sunlight in summer, use of sunbeds and sunlamps, the number of moles (nevi) on the body (described pictorially as none, few, some, many), freckling in childhood and adulthood, and were asked to have someone count the number of moles on their back.

MC1R genotyping

Genomic DNA was isolated from whole blood, or from saliva where whole blood could not be obtained (n=15), by a standard salting out procedure. DNA was amplified using standard PCR and a series of three primer sets as follows:

MC1R-F1: 5'-CAG CAC CAT GAA CTA AGC AGG ACA CCT G-3'

MC1R-R1: 5'-CCA GCA TAG CCA GGA AGA AGA CCA CGA G-3'

MC1R-BF1: 5'-TGC AGC AGC TGG ACA ATG-3'

MC1R-BF2: 5'-AGG ATG GTG AGG GTG ACA GC-3'

MC1R-F2: 5'-TGG GTG GCC AGT GTC GTC TTC AGC A-3'

MC1R-R2: 5'-AAG GGT CCG CGC TTC AAC ACT TTC AGA G-3'

resulting in overlapping amplicons of 671bp, 400bp and 610bp respectively.

PCR products were purified using ExoSap IT (USB Products Affymetrix Inc.), then sent to the Australian Genome Research Facility Ltd for bidirectional sequencing using the 96-capillary 3730xl DNA Analyzer (Applied Biosystems). Variants to the consensus sequence NCBI NM_002386 were identified using BioManager alignment software supplied by the Australian National Genomic Information Service.[15] Sequence chromatograms were also analysed using the DNA variant analysis software Mutation Surveyor (SoftGenetics LLC). Any novel variants identified in either analysis were verified visually.

MC1R classification

Following Raimondi *et al*[3] and Beaumont *et al*,[16] we classified variants D84E, R142H, R151C, I155T, R160W, D294H as `R' variants and all other variants excluding synonymous changes and non-coding changes as `r' variants. R variants have been shown to be strongly associated with the presence of `red hair colour phenotype' (red hair, fair skin, freckling, poor sun sensitivity), whereas r variants generally have a relatively weak association with red-hair colour phenotype.[3] Although the rarer R142H and I155T alleles have not been consistently classified as R variants by previous studies, we classified them *a priori* as R variants for this analysis because other studies have found they were associated with `red hair colour phenotype'[3, 16–18] and are functionally important.[16, 19, 20]

Statistical analysis

Relative risks for melanoma associated with variants and genotypes were estimated by odds ratios (OR) using two types of designs and analysis: 1) unconditional logistic regression comparing cases with population-controls and spouse or friend-controls combined (`case-unrelated-control analysis'), and 2) conditional logistic regression comparing cases with their sibling control/s (`casesibling-control analysis'). For 1), population-controls and spouse or friend-controls were combined into one control group; the *MC1R* genotype frequencies did not significantly differ between these two control groups and we have previously shown that associations between phenotypic risk factors and melanoma were similar when either control group or both groups were used.[14] For all variants, ORs and their corresponding 95% confidence intervals (CIs) were calculated as the increased log odds of melanoma associated with each additional variant allele. The models estimating the OR `per R allele' and `per r allele' were mutually adjusted for one another; i.e. the presented estimates come from a fitted model including both factors. ORs were also calculated for genotype categories that were mutually exclusive, with the reference group comprising only those carrying consensus wildtype alleles.

We excluded participants: 1) whose DNA was unable to be extracted from saliva (1 case, 1 population-control); 2) ≥ 45 years at interview (29 spouse or friend controls, 57 sibling controls); 3) with unknown ethnicity or who reported not having exclusive European ancestry (18 cases, 20 population-controls, 9 spouse or friend controls, 16 sibling controls); and 4) for whom there were missing data for age, lifetime total sun exposure or `pigmentation score' (see below) (13 cases, 3 population-controls, 5 spouse or friend controls, 6 sibling controls). For the case-sibling-control analysis, we also excluded 237 (42%) cases without a suitable sibling control and 26 (5%) siblings whose matched case had been excluded. Therefore, the case-unrelated-control analysis had 565 cases and 409 controls (196 population-controls and 213 spouse or friend controls) and the casesibling-control analysis had 328 cases (being a subset of the 565 cases above) and 518 matched sibling controls (197 cases had one sibling control, 90 cases had two sibling controls, and 41 cases each had three or more sibling controls, up to a maximum of six).

In all fitted models we adjusted for age (as a quadratic), sex, city of recruitment (Brisbane, Sydney, Melbourne), and self-reported European ancestry (British, northern European, southern European, eastern European, mixed European, other/unknown). The European ancestry restriction and adjustment were to account for the different *MC1R* allele frequencies across ethnic groups and within Europe.[21, 22] To determine the extent to which the associations between *MC1R* variants and melanoma risk were independent of self-reported phenotypic pigmentation, we adjusted for `pigmentation score', a continuous variable summarizing the contribution of several correlated, categorical phenotypic variables, as used elsewhere.[23] The variable was created by fitting a logistic regression model with melanoma (case-control) status as the outcome and ability to tan (repeated exposure), propensity to sunburn, skin colour, eye colour, hair colour and childhood freckling as predictors. The predicted probability of melanoma for each individual, based on the beta-values from the logistic regression model, was used as their pigmentation score. A higher score indicates a more sun-sensitive phenotype. Pigmentation scores were created separately for the case-unrelated-control and case-sibling analyses. We also examined the influence on estimates of genetic associations after adjustment for lifetime total sun exposure (quartiles), number of nevi (none, few, some, many), lifetime blistering sunburns (none, ≤ 8, > 8), and sunbed use (0, 1–10, >10 lifetime sessions). We estimated the proportion of familial aggregation of melanoma explained by *MC1R* variants, pigmentation and nevi by examining the percent change in the log OR estimate for the association of confirmed family history with melanoma after adjusting for these variables. This is equivalent to the percent change in variance of a presumed underlying, normally distributed, multiplicative “polygenic” risk.[24]

We tested whether the associations between *MC1R* variants and melanoma risk differed by sex, age, phenotypic characteristics, number of nevi, sun exposure, ambient UV irradiance, lifetime sunburns, sunbed use, family history of melanoma, Breslow thickness, and body site. *P*-values for interaction were obtained by fitting a product term between *MC1R* (per R allele or per r allele) and each stratification variable. For body site and Breslow thickness, we used multinomial logistic regression, and compared heterogeneity in risk estimates for trend across case subgroups to obtain P-values for interaction.

We used χ^2 tests to check whether *MC1R* variants were not in Hardy-Weinberg equilibrium for both control groups. Data were analysed using SAS version 9.2 (SAS Institute, Cary NC) and nominal statistical significance was inferred at two-sided $P < 0.05$.

RESULTS

Descriptive characteristics of cases and control groups

Cases and sibling controls were of similar sex, age and ancestry (Table 1). Cases and unrelated controls were of similar sex but cases were more likely than unrelated controls to be younger and of British ancestry. Cases had higher pigmentation scores than both unrelated controls and sibling controls.

MC1R variants

The prevalence of having at least one R or r variant was 86% of cases, 73% of unrelated controls and 81% of sibling controls (Table 2). Departure from Hardy-Weinberg equilibrium was evident for D294H ($P < 0.001$) for unrelated controls and for D84E ($P = 0.01$), I155T ($P = 0.004$) and R160W ($P = 0.004$) for sibling controls; for all deviations we observed more homozygous carriers and less heterozygous carriers of the variant allele than expected.

Association of MC1R genotypes with phenotypic characteristics among controls

For controls combined, carrying any R variant was strongly associated with red hair, or fair skin (online only Supplementary Table 1). Carrying any r variant (in the absence of R variants) was weakly associated with these phenotypes. Nearly all (94%) controls with red hair carried at least one R allele compared to 44% of those without red hair. Each R variant was positively associated with red hair colour; the `per allele' ORs (95% CI) for red hair for unrelated and sibling controls combined were 2.08 (0.91–4.72) for D84E, 4.33 (2.81–6.69) for R142H, 4.35 (2.80–6.77) for R151C, 2.44 (1.06–5.60) for I155T, 4.33 (2.81–6.69) for R160W, and 3.00 (1.55–5.82) for D294H. Carriers of V60L, V92M or R163Q alleles were less likely to have red hair colour (all ORs < 1).

Minimally adjusted associations of MC1R variants and genotypes with melanoma

R151C and I155T were associated with an approximately two-fold increase melanoma risk per allele from both case-unrelated-control and case-sibling-control comparisons (Table 3). From the case-unrelated-control analysis, the ORs per allele for the other R variants ranged from 1.11 to 1.25 and the confidence intervals included the null value. From the case-sibling-control analysis, R160W and D84E were also associated with approximately two-fold increased risk, but there was no apparent association for D294H or R142H.

When analysed in six genotype categories (from wildtype only to two R alleles), people with (any) two R alleles had almost five times the estimated risk of melanoma of those carrying wildtype alleles only (i.e. no variant alleles) (Table 4). Carrying two or more r alleles, one R and no r alleles, or one R and one r allele, was associated with a 2- to 4-fold increased risk of melanoma. Carrying one r and no R alleles was weakly positively associated with risk.

When all the R and r variants were combined, each variant allele carried was associated with an estimated doubling of melanoma risk from the case-unrelated-control analysis and 66% higher risk from the case-sibling-control analysis (Table 4). For R variants combined, each R allele carried approximately doubled the risk of melanoma and this was similar for both control group analyses. For r variants combined, each additional r variant increased risk by an estimated 25% (case-sibling-control comparison) and 69% (case-unrelated-control comparison). The *P* value for heterogeneity comparing the `per allele' OR for the combined R variants with that for the combined r variants was 0.10 for the case-unrelated-control analysis and 0.05 for the case-sibling-control analysis.

Associations of MC1R variants and genotypes with melanoma adjusted for pigmentation, nevi and UV exposure

Adjustment for pigmentation score moderately attenuated the associations of R variants with melanoma: the overall per allele OR fell from 2.23 to 1.68 (lower confidence bound 1.30) for the case-sibling-control comparison and from 2.06 to 1.74 (lower confidence bound 1.23) for the case-unrelated-control comparison (Tables 3 and 4). This adjustment slightly attenuated the per r allele association. Additional adjustment for lifetime total sun exposure, lifetime blistering sunburns, sunbed use and number of nevi resulted in minimal change to OR estimates for most r variants, but strengthened associations for several R variants: the overall per R allele OR increased to 1.93 and 1.81 respectively for the two types of analysis, resulting in approximately 18% attenuation from the minimally adjusted log OR estimates (Tables 3 and 4). The change in OR estimates was mainly due to inclusion of number of nevi in the model.

Familial aggregation of melanoma explained by MC1R

Having a first degree relative with confirmed melanoma approximately doubled the risk of melanoma compared to having no family history; OR 2.08, 95% CI 1.21–3.60 (Table 5). After adjusting for *MC1R*, this OR fell to 1.79 (95% CI 1.02–3.14), suggesting that *MC1R* variants explained about 21% of the familial aggregation of melanoma. Addition of pigmentation and nevi to the model also weakened the family history association, and adjustment for all factors combined attenuated the association by about 37%.

Associations of MC1R variants with melanoma, stratified by demographic factors, phenotype, sun exposure and melanoma characteristics

The association of *MC1R* variants, analysed as any R or r allele, with melanoma risk was stronger for men than for women; $P_{\text{interaction}}$ 0.005 for the case-unrelated-control comparison (Table 6). There was a stronger association of *MC1R* variants with melanoma in those with none or few nevi than in those with some or many nevi ($P_{\text{interaction}}$ <0.001 for the case-unrelated-control comparison and 0.05 for the case-sibling-control comparison). The ORs were higher for participants with higher levels of childhood total sun exposure ($P_{\text{interaction}}$ 0.008 for the case-unrelated-control comparison). *MC1R* variants were more strongly associated with superficial spreading and nodular melanoma than with other histological subtypes of melanoma in the case-unrelated-control comparison ($P_{\text{interaction}}$ 0.03) but no difference was apparent for the case-sibling-control comparison. Adjustment of

these stratified analyses for pigmentation score resulted in minimal change to the interaction *P*-values (data not shown). More detailed results showing the per R allele and per r allele stratified associations with melanoma are shown in online only Supplementary Table 2 to facilitate comparison and meta-analysis with other studies.

DISCUSSION

Our results show that *MC1R* variants are an important determinant of early-onset melanoma. A high proportion of the population carry a *MC1R* variant; 73% of unrelated controls had at least one R or r variant, 40% carried an R variant, and 43% an r variant. Risk of melanoma approximately doubled for each R variant allele carried and increased by 23–55% for each r variant allele, after adjusting for phenotype and UV exposure. We estimate that approximately 21% of the increased risk of early-onset melanoma associated with family history is explained by *MC1R* variants.

The allele frequencies for unrelated control subjects in our study were similar to those reported previously in Australia, United Kingdom and the United States.[8, 19, 22] The prevalence of variants in our early-onset cases (86%) is the same as for cases with multiple primary melanoma in the population-based GEM study and similar to that for single primary melanoma (83%).[25] A recent case-control study of white, non-Hispanic participants from the United States reported prevalences of 78% for cases and 71% for controls.[4] A meta-analysis of studies conducted in different countries reported lower weighted prevalence estimates for cases: 28% for R and 33% for r variants.[5] These lower estimates could be due to most previous studies genotyping only a selection of variants and to differences in ethnicity or genetic susceptibility of the study populations.

The magnitude of associations between *MC1R* variants and melanoma in this study are similar to pooled effect estimates from published meta-analyses.[3, 5] Williams *et al*[5] reported a `per person' (heterozygotes and homozygotes combined) pooled OR of 2.44 (95% CI 1.72–3.45) for R variants combined and 1.29 (95% CI 1.10–1.51) for r variants combined, although the classification varied from our study because we included I155T and R142H as R variants and all non-synonymous non-R variants as r variants. I155T and R142H have a low prevalence and when we classified them as r variants in a sensitivity analysis, our results changed minimally. The classification of R and r variants is based mainly on association with `red hair colour phenotype', although R variants have also been found to have a stronger association with melanoma than r variants, and our data generally support that difference. We observed evidence of a `dose-response' relationship; risk was 2–3 times higher for those with two or more r variants and about 4–5 times higher for those with two R variants, compared to those with no *MC1R* variants. Some studies[8, 26] have observed a similar dose-response for R variants, but other studies have reported weaker dose-response patterns.[4, 25] Our results are consistent with the hypothesis that pigmentation only partially mediates the association between *MC1R* variants and melanoma and that other biological pathways must be involved,[4, 7–9] since adjustment for pigmentation factors only partly attenuated the risk estimates for R variants and had minimal influence on r variant risk estimates.

We observed a stronger association of *MC1R* variants with melanoma risk for those with none or few nevi than for those with some or many nevi, but no important differences for other phenotypic characteristics. Contrariwise, a meta-analysis by Kanetsky *et al* of (relatively few) studies reporting stratum-specific associations showed generally stronger associations between *MC1R* variants and melanoma for those with `protective' phenotypes such as dark hair, eyes or skin, but slightly weaker associations for those with low nevus counts.[4] We found some evidence too of higher ORs with higher sun exposure. In the meta-analysis,[4] the association of R variants with melanoma was similar for those with high and low recreational sun exposure, but for r variants the association was stronger for those with high sun exposure (OR 1.6) than for those with low sun exposure (OR 1.1). We observed stronger associations for men than for women; to our knowledge, other studies have not reported sex differences. It is likely that some of our observed interactions are purely chance findings, given the number of interactions tested and occasional inconsistent results for case-unrelated-control and case-sibling-control comparisons. Unlike the Genes, Environment and Melanoma (GEM) study,[23] we did not observe a three-way interaction between R variants, sun exposure and body site, but we had limited statistical power to detect complex interactions.

A limitation of our study was the self-reported measures of phenotypic characteristics and UV exposures, which may have led to misclassification and residual confounding. Also, we created a continuous `pigmentation score' variable to summarize the contribution of several correlated, categorical phenotypic variables, but we acknowledge that this may not fully account for the effects of each phenotypic variable. The departure from Hardy-Weinberg equilibrium that we observed for some *MC1R* variants could indicate preferential participation of controls with sun sensitive phenotypes, or perhaps less sensitivity of the genotyping technique for heterozygosity for those variants.

A strength of the population-based, case-control-family study design was the ability to conduct analyses using unrelated controls in addition to sibling controls. The associations with melanoma that we observed for the combined R variants, combined r variants and genotype categories were quite similar and consistent for the two types of analyses. As it becomes more difficult to recruit population-based controls,[27] the use of other control groups such as spouse, friend or sibling controls is becoming more appealing. Advantages of sibling controls include avoiding population stratification (confounding by ethnicity), potentially higher participation, lower costs, and efficiency when studying gene-environment interactions.[14, 28–32] However, it is a disadvantage that cases who do not have an eligible and willing sibling control are excluded from the analysis, influencing the statistical power.[30]

In conclusion, we found that *MC1R* variants are associated with increased risk of early-onset melanoma, and these associations appeared to be mediated through pigmentary and non-pigmentary pathways. There was some evidence that associations between *MC1R* and melanoma were stronger for certain subgroups including men and those with none or few nevi. Measuring *MC1R* genotype could help us to better target risk reduction, screening or surveillance strategies for melanoma, but further evaluation of its utility for these purposes is required to guide future clinical and public health applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MC1R	melanocortin-1-receptor gene
OR	odds ratio
CI	confidence interval

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Table 1

Characteristics of cases, unrelated controls and sibling controls in the Australian Melanoma Family Study

Characteristic	Case-unrelated-control analysis				Case-sibling-control analysis			
	Cases (n=565)		Unrelated controls[a] (n = 409)		Cases (n=328)		Sibling controls (n=518)	
	N	%	N	%			N	%
Sex								
Male	208	(37)	168	(41)	119	(36)	200	(39)
Female	357	(63)	241	(59)	209	(64)	318	(61)
P-value [c]				0.2				0.5
Age at diagnosis/interview (years)[b]								
18–24	63	(11)	27	(7)	36	(11)	42	(8)
25–29	110	(19)	52	(13)	58	(18)	90	(17)
30–34	176	(31)	117	(29)	106	(32)	144	(28)
34–39	216	(38)	148	(36)	128	(39)	152	(29)
40–44	0	(0)	65	(16)	0	(0)	90	(17)
P-value [c]				<0.001				0.7
European ancestry								
British	386	(68)	239	(58)	232	(71)	366	(71)
Northern European	31	(5)	13	(3)	13	(4)	18	(3)
Southern European	21	(4)	25	(6)	10	(3)	19	(4)
Eastern European	5	(1)	7	(2)	2	(1)	5	(1)
Mixed European	114	(20)	115	(28)	69	(21)	104	(20)
Other/unknown	8	(1)	10	(2)	2	(1)	6	(1)
P-value [c]				0.003				0.7
Pigmentation score[d]								
1st quartile (low-risk)	49	(9)	103	(25)	25	(8)	130	(25)
2nd quartile	130	(23)	102	(25)	59	(18)	129	(25)
3rd quartile	146	(26)	102	(25)	76	(23)	130	(25)

Characteristic	Case-unrelated-control analysis				Case-sibling-control analysis			
	Cases (n=565)		Unrelated controls[a] (n = 409)		Cases (n=328)		Sibling controls (n=518)	
4th quartile (high-risk)	240	(42)	102	(25)	168	(51)	129	(25)
P-value [c]			<0.001				<0.001	

[a] Unrelated controls include 196 population controls and 213 spouse/friend controls.

[b] All cases were < 40 years at diagnosis and all population controls were < 40 years when ascertained. Cases and controls could be up to age 44 years at interview for this analysis.

[c] *P*-values for difference in proportions between cases and controls for each analytical group. For the case-unrelated-control analysis the *P*-values are from Pearson chi-square tests. For case-sibling-control analysis they are Wald *P*-values from conditional logistic regression models matched on family.

[d] A continuous variable summarizing the contribution of several correlated, categorical phenotypic variables. A higher score indicates a more sun-sensitive phenotype.

Table 2

Allele and genotype frequencies for *MC1R* variants observed in cases and controls

Nucleotide change	Protein change	Case	Unrelated controls[a]	Sibling-controls
		N	N	N
Number of participants		565	409	518
Allele frequency (%) [b]		%	%	%
R variants				
g.252C>A	D84E	2.5	1.7	2.8
g.425G>A	R142H	1.2	1.0	1.5
g.451C>T	R151C	16.9	7.5	12.5
g.464T>C	I155T	2.3	1.3	2.6
g.478C>T	R160W	10.4	8.7	9.3
g.880G>C	D294H	3.7	3.3	3.2
r variants				
g.178G>T	V60L	12.3	10.3	11.4
g.274G>A	V92M	9.6	7.7	8.0
g.488G>A	R163Q	4.6	4.5	4.4
Insertion/deletion[c]		0.6	0.5	0.8
Rare nonsynonymous[d]		1.2	1.2	1.4
Silent changes				
g.942A>G	T314T	12.2	8.1	10.5
Other synonymous[e]		1.2	0.4	0.8
Non-coding regions[f]		0.6	0.1	0.0
Genotype frequency (%) [g]				
wildtype consensus alleles only		13.8	27.1	19.1
1 r and 0 R alleles		19.3	26.4	22.4
≥ 2 r alleles and 0 R alleles		8.5	6.4	5.2
1 R and 0 r alleles		23.2	23.5	23.9
1 R and 1 r allele		19.5	9.8	18.7
2 R alleles		15.8	6.8	10.6

[a] Unrelated controls include population controls and spouse/friend controls

[b] As a proportion of all alleles genotyped. Number of alleles is double the number of individuals as *MC1R* is autosomal.

[c] Insertion-deletion variants include: 86insA, 537insC

[d] Rare non-synonymous variants include: Q23H, R34Q, V38M,F45L, A64T, S83P, T95M, G104S, R109W, I120T, V122M, S131N, D141V, R142C, Y152N, Y152X, V156A, T19I, F196L, R213W, A218T, A222T, P230L, T242I, N279L, L282F, R306H

[e] Rare synonymous variants where the minor allele frequency in controls was <1% include: R34R, N56N, G89G, C133C, A139A, R151R, A164A, Y182Y, H185H, R213R, G239G, Q233Q, Q234Q, I264I, F300F, T308T, S316S

[f] Non-coding regions include: in the 3'UTR: g.−20C>T; in the intron beyond the stop codon: 954+2C>T, 954+13C>T, 954+21A>C, 954+69T>G, 954+104C>G

[g] As a proportion of all individuals genotyped. The categories are mutually exclusive. Silent changes (i.e. changes that are synonymous or occur in non-coding regions) are counted as consensus alleles. 1 person carried 3 r alleles but nobody carried > 2 R alleles.

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Table 3

Associations of *MC1R* variants with melanoma, with and without adjustment for pigmentation and sun exposure, for case-unrelated-control and case-sibling-control comparisons

MC1R variant	Case-unrelated-control[a] comparison				Case-sibling-control comparison			
	Cases / controls	OR[b] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)	Cases / controls	OR[c] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)
R variants								
D84E								
D/D	537/395	1.00	1.00	1.00	307/491	1.00	1.00	1.00
D/E	28/14	1.25 (0.64–2.46)	0.96 (0.48–1.91)	1.21 (0.59–2.51)	21/25	1.88 (0.76–4.67)	1.60 (0.63–4.08)	1.94 (0.70–5.35)
E/E	0/0				0/2			
R142H								
R/R	551/401	1.00	1.00	1.00	319/502	1.00	1.00	1.00
R/H	14/8	1.22 (0.48–3.09)	0.82 (0.31–2.15)	0.74 (0.27–2.00)	9/16	0.99 (0.26–3.84)	1.07 (0.26–4.41)	1.35 (0.29–6.26)
H/H	0/0				0/0			
R151C								
R/R	392/350	1.00	1.00	1.00	225/398	1.00	1.00	1.00
R/C	155/57	2.57 (1.86–3.56)	1.99 (1.42–2.78)	2.12 (1.49–3.00)	92/110	1.70 (1.12–2.60)	1.49 (0.96–2.31)	1.58 (0.99–2.52)
C/C	18/2				11/10			
I155T								
I/I	539/398	1.00	1.00	1.00	311/493	1.00	1.00	1.00
I/T	26/11	2.10 (0.97–4.53)	1.80 (0.81–4.03)	1.91 (0.83–4.38)	17/23	2.48 (0.76–8.15)	2.11 (0.63–7.13)	2.31 (0.65–8.15)
T/T	0/0				0/2			
R160W								
R/R	454/342	1.00	1.00	1.00	259/432	1.00	1.00	1.00
R/W	104/63	1.18 (0.85–1.63)	0.95 (0.68–1.34)	1.03 (0.73–1.46)	63/76	1.95 (1.19–3.20)	1.69 (1.01–2.81)	1.61 (0.93–2.77)
W/W	7/4				6/10			
D294H								
D/D	523/385	1.00	1.00	1.00	308/485	1.00	1.00	1.00
D/H	42/21	1.11 (0.67–1.84)	0.85 (0.50–1.46)	0.96 (0.54–1.70)	20/33	0.89 (0.40–1.99)	0.79 (0.35–1.76)	0.61 (0.25–1.50)

MC1R variant	Case-unrelated-control[a] comparison				Case-sibling-control comparison			
	Cases / controls	OR[b] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)	Cases / controls	OR[c] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)
H/H	0/3				0/0			
r variants								
V60L								
V/V	436/330	1.00	1.00	1.00	263/406	1.00	1.00	1.00
V/L	119/74	1.33 (0.98–1.80)	1.36 (0.99–1.86)	1.30 (0.94–1.79)	61/106	0.73 (0.47–1.13)	0.68 (0.43–1.09)	0.66 (0.40–1.10)
L/L	10/5				4/6			
V92M								
V/V	464/349	1.00	1.00	1.00	274/438	1.00	1.00	1.00
V/M	94/57	1.21 (0.86–1.70)	1.27 (0.90–1.79)	1.31 (0.92–1.86)	51/77	1.18 (0.73–1.91)	1.23 (0.75–2.02)	1.22 (0.72–2.08)
M/M	7/3				3/3			
R163Q								
R/R	514/369	1.00	1.00	1.00	297/472	1.00	1.00	1.00
R/Q	50/37	0.82 (0.53–1.26)	0.88 (0.56–1.36)	0.76 (0.48–1.19)	31/46	1.21 (0.64–2.29)	1.22 (0.62–2.38)	1.22 (0.61–2.45)
Q/Q	1/3							
Insertion / deletion[f]								
con/con	558/405	1.00	1.00	1.00	326/510	1.00	1.00	1.00
con/var	7/4	1.94 (0.50–7.56)	1.30 (0.33–5.16)	0.96 (0.24–3.88)	2/8	0.44 (0.07–2.92)	0.58 (0.08–4.31)	0.70 (0.10–4.99)
var/var	0/0				0/0			
Other nonsynonymous[f]								
con/con	553/399	1.00	1.00	1.00	321/505	1.00	1.00	1.00
con/var	11/10	1.17 (0.50–2.78)	1.08 (0.43–2.71)	1.29 (0.50–3.28)	6/12	0.99 (0.24–3.99)	1.11 (0.27–4.61)	1.67 (0.35–8.05)
var/var	1/0				1/1			

[a] Unrelated controls include population controls and spouse/friend controls.

[b] Unconditional logistic regression, adjusted for age, sex, city of recruitment, and European ancestry. ORs were calculated as the risk associated with each additional variant allele carried.

[c] Conditional logistic regression, matched on family and adjusted for age and sex. ORs were calculated as the risk associated with each additional variant allele carried.

[d] Adjusted for the factors in footnote b, plus pigmentation score (continuous), which is a continuous variable derived from several variables including: usual skin response to sun exposure (ability to tan), propensity to sunburn, skin colour, eye colour, hair colour and childhood freckling.

[e] Adjusted for the factors in footnote b, plus pigmentation score (continuous), lifetime total sun exposure (quartiles), nevus density (4 categories), lifetime blistering sunburns (none, ≤ 8, > 8), lifetime number of sunbed sessions (0, 1–10, >10).

[f] See Table 2 footnotes describing which variants are included in this category

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Table 4

Associations of *MC1R* genotypes for grouped variants with melanoma, with and without adjustment for pigmentation and sun exposure, for case-unrelated-control and case-sibling-control comparisons

MC1R variant	Case-unrelated-control[a] comparison				Case-sibling-control comparison			
	Cases / controls	OR[b] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)	Cases / controls	OR[c] (95% CI)	OR adj for pigmentation[d] (95% CI)	OR adj for pigmentation, nevi & sun exposure[e] (95% CI)
MC1R categories[f]								
wildtype only	78/112	1.00	1.00	1.00	48/99	1.00	1.00	1.00
1 r and 0 R alleles	108/105	1.60 (1.05–2.42)	1.45 (0.95–2.22)	1.39 (0.89–2.16)	54/115	1.16 (0.62–2.16)	1.14 (0.59–2.18)	1.43 (0.72–2.84)
≥2 r alleles and 0 R alleles	49/28	2.68 (1.48–4.84)	2.33 (1.27–4.25)	2.26 (1.21–4.20)	25/28	2.70 (0.99–7.40)	2.83 (0.98–8.14)	2.94 (0.95–9.04)
1 R and 0 r alleles	128/94	1.97 (1.30–2.99)	1.69 (1.11–2.59)	1.70 (1.09–2.65)	82/117	2.17 (1.18–3.97)	1.85 (1.00–3.45)	1.87 (0.97–3.60)
1 R and 1 r allele	113/42	4.25 (2.60–6.95)	3.13 (1.88–5.20)	3.54 (2.08–6.05)	64/104	2.21 (1.12–4.33)	1.59 (0.78–3.24)	1.67 (0.77–3.59)
2 R alleles	89/28	4.89 (2.84–8.43)	2.42 (1.33–4.42)	3.42 (1.81–6.47)	55/55	4.52 (2.13–9.62)	3.45 (1.59–7.47)	4.16 (1.82–9.50)
Combined variants								
per r allele[g]	565/409	1.69 (1.33–2.13)	1.53 (1.21–1.95)	1.55 (1.20–1.99)	328/518	1.25 (0.88–1.79)	1.18 (0.81–1.71)	1.23 (0.82–1.83)
per R allele[g]	565/409	2.23 (1.77–2.80)	1.68 (1.30–2.16)	1.93 (1.47–2.52)	328/518	2.06 (1.47–2.88)	1.74 (1.23–2.48)	1.81 (1.24–2.65)
Per R or r allele	565/409	1.96 (1.61–2.38)	1.60 (1.30–1.96)	1.71 (1.38–2.12)	328/518	1.66 (1.25–2.20)	1.47 (1.09–1.97)	1.52 (1.10–2.10)

[a] Unrelated controls include population controls and spouse/friend controls.

[b] Unconditional logistic regression, adjusted for age, sex, city of recruitment, and European ancestry. ORs were calculated as the risk associated with each additional variant allele carried.

[c] Conditional logistic regression, matched on family and adjusted for age and sex. ORs were calculated as the risk associated with each additional variant allele carried.

[d] Adjusted for the factors in footnote b, plus pigmentation score (continuous), which is a continuous variable derived from several variables including: usual skin response to sun exposure (ability to tan), propensity to sunburn, skin colour, eye colour, hair colour and childhood freckling.

[e] Adjusted for the factors in footnote b, plus pigmentation score (continuous), lifetime total sun exposure (quartiles), nevus density (4 categories), lifetime blistering sunburns (none, ≤ 8, > 8), lifetime number of sunbed sessions (0, 1–10, >10).

[f] The categories are mutually exclusive. Silent changes (i.e. changes that are synonymous or occur in non-coding regions) are counted as consensus alleles.

[g] The models estimating the OR per `R' allele and per `r' allele were mutually adjusted in order to estimate their independent effects. r variants exclude silent changes (i.e. changes that are synonymous or occur in non-coding regions).

Table 5

The amount of familial aggregation of melanoma explained by *MC1R* variants, pigmentation and nevi, from case-unrelated-control analyses

Confirmed family history of melanoma	Cases / controls[a]	OR[b]	OR adj for *MC1R*[c]	OR adj for pigmentation and nevi[d]	OR adj for all factors[e]
1st degree relative					
No	509/388	1.00	1.00	1.00	1.00
Yes	56/21	2.08 (1.21–3.60)	1.79 (1.02–3.14)	1.73 (0.98–3.07)	1.59 (0.89–2.84)

[a] Population controls and spouse/friend controls.

[b] Unconditional logistic regression, adjusted for age, sex, city of recruitment, and European ancestry.

[c] Adjusted for the factors in footnote b, plus *MC1R* as per R allele and per r allele variables.

[d] Adjusted for the factors in footnote b, plus pigmentation score (continuous) and nevus density (4 categories).

[e] Adjusted for the factors in footnote b, plus *MC1R*, pigmentation score (continuous) and nevus density (4 categories).

Table 6

Associations of *MC1R* variants (any R or r allele) with melanoma, by demographic factors, phenotype, sun exposure and melanoma characteristics

	Case-unrelated-control analysis		Case-sibling-control analysis	
Stratification variable	**Cases / controls**	**OR[b] (95% CI) per any R or r allele[c]**	**Cases / controls**	**OR[d] (95% CI) per any R or r allele[c]**
None	565/409	1.96 (1.61–2.38)	328/518	1.66 (1.25–2.20)
Sex				
Female	357/241	1.58 (1.25–2.01)	145/203	1.56 (1.01–2.44)
Male	208/168	2.90 (2.04–4.11)	63/78	3.72 (1.71–8.08)
p interaction		0.005		0.1
Age				
18–29	173/79	2.46 (1.61–3.77)	69/80	1.13 (0.58–2.19)
30–44	392/330	1.88 (1.50–2.35)	218/334	1.90 (1.29–2.80)
p interaction		0.5		0.1
Family history of melanoma[e]				
No	509/388	1.97 (1.61–2.42)		
Yes	56/21	1.34 (0.62–2.92)		
p interaction		0.3		
Pigmentation score[f]				
≤ Median	235/248	1.68 (1.26–2.25)	97/202	2.01 (1.17–3.44)
>Median	330/161	1.89 (1.42–2.51)	151/152	1.60 (0.95–2.69)
p interaction		0.6		0.4
Hair colour				
Brown/Black	371/338	1.74 (1.38–2.19)	194/271	1.90 (1.28–2.82)
Red/Blonde/Fair	192/71	1.92 (1.24–2.98)	64/78	1.18 (0.47–2.94)
p interaction		0.9		0.7
Skin colour				
Olive/Brown	55/68	1.95 (1.09–3.50)	18/21	1.27 (0.43–3.75)
Very fair/Fair	507/340	1.93 (1.56–2.39)	268/419	1.66 (1.20–2.29)
p interaction		0.9		0.1
Eye colour				
Hazel/Brown/Black	307/228	1.98 (1.53–2.56)	140/199	1.67 (1.07–2.59)
Blue/Grey/Green	256/177	1.94 (1.43–2.63)	108/172	1.63 (0.98–2.68)
p interaction		0.83		0.5
Usual skin response[g]				
Usually/Always tans	259/238	1.72 (1.31–2.25)	103/131	2.01 (1.17–3.45)
Sometimes/Never tans	304/168	2.36 (1.73–3.21)	119/175	1.78 (1.03–3.07)
p interaction		0.2		0.4
Propensity to sunburn[h]				
Mild/No burn	196/196	1.78 (1.30–2.43)	72/87	1.08 (0.55–2.10)

Stratification variable	Case-unrelated-control analysis		Case-sibling-control analysis	
	Cases / controls	OR[b] (95% CI) per any R or r allele[c]	Cases / controls	OR[d] (95% CI) per any R or r allele[c]
Severe/Painful burn	368/212	1.96 (1.51–2.56)	151/223	2.01 (1.26–3.19)
p interaction		0.7		0.3
Childhood Freckling[i]				
None/Very few	267/240	2.09 (1.55–2.80)	117/152	1.70 (1.00–2.91)
Few/Some/Many	295/168	1.78 (1.33–2.39)	111/160	1.17 (0.68–2.01)
p interaction		0.6		0.9
Number of nevi[j]				
None/Few	172/234	3.07 (2.20–4.27)	78/111	1.86 (0.92–3.73)
Some/Many	391/173	1.54 (1.18–2.01)	148/192	1.34 (0.87–2.07)
p interaction		<0.001		0.05
Childhood total sun exposure				
≤ Median	286/205	1.53 (1.18–1.99)	121/157	1.28 (0.81–2.02)
> Median	279/204	2.61 (1.93–3.51)	94/127	1.97 (1.14–3.42)
p interaction		0.008		0.3
Childhood sunburn causing blisters				
None	366/283	1.74 (1.38–2.21)	173/248	1.66 (1.15–2.39)
Any	196/126	2.47 (1.73–3.52)	57/69	2.44 (0.83–7.22)
p interaction		0.1		0.3
Childhood ambient UV irradiance[k]				
≤ Median	330/207	2.09 (1.58–2.75)	158/248	1.59 (1.06–2.39)
> Median	234/202	1.88 (1.42–2.48)	163/249	1.73 (1.14–2.63)
p interaction		0.7		1.0
Sunbed use				
None	432/339	2.10 (1.68–2.62)	228/341	1.52 (1.08–2.14)
Any	130/68	1.52 (0.99–2.33)	27/35	1.85 (0.66–5.13)
p interaction		0.1		0.8
Body site of melanoma				
Head or neck	74/409	1.85 (1.28–2.66)	40/65	1.50 (0.60–3.73)
Trunk	190/409	1.75 (1.36–2.25)	114/185	1.40 (0.88–2.23)
Arms	121/409	1.93 (1.44–2.60)	68/104	1.64 (0.82–3.28)
Legs	168/409	2.40 (1.82–3.15)	100/153	2.67 (1.45–4.93)
p interaction		0.2		0.6
Breslow thickness				
≤ 0.75 mm	367/409	1.81 (1.47–2.23)	222/342	1.60 (1.14–2.25)
> 0.75 mm	195/409	2.27 (1.75–2.93)	104/173	1.72 (1.02–2.89)
p interaction		0.09		0.8
Histological subtype[l]				
Superficial spreading	326/409	1.97 (1.58–2.45)	13/21	2.74 (0.48–15.71)
Nodular	25/409	3.79 (1.98–7.25)	108/156	1.33 (0.78–2.27)

	Case-unrelated-control analysis		Case-sibling-control analysis	
Stratification variable	**Cases / controls**	**OR[b] (95% CI) per any R or r allele[c]**	**Cases / controls**	**OR[d] (95% CI) per any R or r allele[c]**
Other	16/409	0.98 (0.49–1.97)	8/15	1.26 (0.03–59.17)
Not otherwise specified	198/409	1.93 (1.50–2.49)	199/326	1.78 (1.24–2.55)
p interaction		0.03		0.7

[a] Unrelated controls include population controls and spouse/friend controls

[b] Unconditional logistic regression, adjusted for age, sex, city of recruitment, and European ancestry.

[c] ORs were calculated as the risk associated with each additional variant allele carried. Excludes silent changes (i.e. changes that are synonymous or occur in non-coding regions).

[d] Conditional logistic regression, matched on family and adjusted for age and sex.

[e] Confirmed family history of melanoma in 1st degree relatives

[f] A continuous variable summarizing the contribution of several correlated, categorical phenotypic variables. A higher score indicates a more sun-sensitive phenotype

[g] Usual skin response to sun exposure: Never or sometimes tans, usually or always burns *cf.* Usually or always tans, never or sometimes burns

[h] Skin response to exposure to bright sunlight for the first time in summer without protection

[i] Based on a 6-level picture scale of the face

[j] Number of nevi covering the body based on a 4-level picture scale

[k] Erythemally weighted ambient UV irradiance (kJ/m^2) derived from residence history and data from satellite observations

[l] `Other' histological subtypes include malignant melanoma, regressing; lentigo maligna melanoma; acral lentiginous melanoma; and desmoplastic melanoma.