Br J Dermatol 2023; **188**:770–776
https://doi.org/10.1093/bjd/ljad041
Advance access publication date: 5 March 2023

BJD
British Journal of Dermatology
Epidemiology

The *MC1R* r allele does not increase melanoma risk in *MITF* E318K carriers

Courtney K. Wallingford,[1] Anastassia Demeshko,[1] Asha Krishnankutty Krishnakripa,[1] Darren J. Smit,[1] David L. Duffy,[1,2] Brigid Betz-Stablein,[1,2] Annette Pflugfelder,[3] Kasturee Jagirdar,[1,4] Elizabeth Holland,[5] Graham J. Mann,[6,7] Clare A. Primiero,[1] Tatiane Yanes,[1] Josep Malvehy,[8,9] Cèlia Badenas,[9,10] Cristina Carrera,[8,9] Paula Aguilera,[8,9] Catherine M. Olsen,[1,2] Sarah V. Ward,[11] Nikolas K. Haass,[1] Richard A. Sturm,[1] Susana Puig,[8,9] David C. Whiteman,[2] Matthew H. Law,[12,13,14] Anne E. Cust,[5,6] Miriam Potrony,[9,10] H. Peter Soyer[1,15] and Aideen M. McInerney-Leo[1]

[1]Frazer Institute, University of Queensland, Dermatology Research Centre, Brisbane, Australia
[2]QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, Queensland, Australia
[3]Center of Dermato-Oncology, Department of Dermatology, University of Tübingen, Tübingen, Germany
[4]Biochemistry and Molecular Biology Department, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[5]The Daffodil Centre, The University of Sydney, a joint venture with Cancer Council NSW, Sydney, Australia
[6]The Melanoma Institute Australia, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia
[7]John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia
[8]Dermatology Department, Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona, Spain
[9]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain
[10]Biochemistry and Molecular Genetics Department, Hospital Clínic de Barcelona, IDIBAPS, Barcelona, Spain
[11]School of Population and Global Health, The University of Western Australia, Perth, WA, Australia
[12]Statistical Genetics, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Herston, QLD, 4006, Australia
[13]School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Queensland, Australia
[14]School of Biomedical Sciences, University of Queensland, Brisbane, Australia
[15]Dermatology Department, Princess Alexandra Hospital, Brisbane, Australia

Correspondence: Aideen M. McInerney-Leo. Email: a.mcinerney@uq.edu.au

Linked Article: Bruno *Br J Dermatol* 2023; **188**:696–697.

Abstract

Background Population-wide screening for melanoma is not cost-effective, but genetic characterization could facilitate risk stratification and targeted screening. Common *Melanocortin-1 receptor* (*MC1R*) red hair colour (RHC) variants and *Microphthalmia-associated transcription factor* (*MITF*) E318K separately confer moderate melanoma susceptibility, but their interactive effects are relatively unexplored.

Objectives To evaluate whether *MC1R* genotypes differentially affect melanoma risk in *MITF* E318K$^+$ vs. E318K$^-$ individuals.

Materials and methods Melanoma status (affected or unaffected) and genotype data (*MC1R* and *MITF* E318K) were collated from research cohorts (five Australian and two European). In addition, RHC genotypes from E318K$^+$ individuals with and without melanoma were extracted from databases (The Cancer Genome Atlas and Medical Genome Research Bank, respectively). χ^2 and logistic regression were used to evaluate RHC allele and genotype frequencies within E318K$^{+/-}$ cohorts depending on melanoma status. Replication analysis was conducted on 200 000 general-population exomes (UK Biobank).

Results The cohort comprised 1165 *MITF* E318K$^-$ and 322 E318K$^+$ individuals. In E318K$^-$ cases *MC1R* R and r alleles increased melanoma risk relative to wild type (wt), $P<0.001$ for both. Similarly, each *MC1R* RHC genotype (R/R, R/r, R/wt, r/r and r/wt) increased melanoma risk relative to wt/wt ($P<0.001$ for all). In E318K$^+$ cases, R alleles increased melanoma risk relative to the wt allele [odds ratio (OR) 2.04 (95% confidence interval 1.67–2.49); $P=0.01$], while the r allele risk was comparable with the wt allele [OR 0.78 (0.54–1.14) vs. 1.00, respectively]. E318K$^+$ cases with the r/r genotype had a lower but not significant melanoma risk relative to wt/wt [OR 0.52 (0.20–1.38)]. Within the E318K$^+$ cohort, R genotypes (R/R, R/r and R/wt) conferred a significantly higher risk compared with non-R genotypes (r/r, r/wt and wt/wt) ($P<0.001$). UK Biobank data supported our findings that r did not increase melanoma risk in E318K$^+$ individuals.

Conclusions RHC alleles/genotypes modify melanoma risk differently in *MITF* E318K$^-$ and E318K$^+$ individuals. Specifically, although all RHC alleles increase risk relative to wt in E318K$^-$ individuals, only *MC1R* R increases melanoma risk in E318K$^+$ individuals. Importantly, in the E318K$^+$ cohort the *MC1R* r allele risk is comparable with wt. These findings could inform counselling and management for *MITF* E318K$^+$ individuals.

Accepted: 18 February 2023

What is already known about this topic?

- *Melanocortin-1 receptor* (*MC1R*) red hair colour (RHC) variants additively increase melanoma risk 1.55–1.93-fold. They also modify penetrance in *CDKN2A* carriers.
- *Microphthalmia-associated transcription factor* (*MITF*) E318K increases melanoma risk 2.37–2.63-fold.
- *MITF* E318K$^+$ combined with an RHC variant is hypothesized to increase melanoma risk.
- Functional research suggests that *MC1R* R/R genotypes are most susceptible to melanocytic cell transformation by forced expression of MITF.
- No studies have explored the interactive effect of E318K and individual *MC1R* RHC variants.

What does this study add?

- In E318K$^+$ individuals, the *MC1R* R allele was associated with increased melanoma risk while the *MC1R* r allele risk was comparable with the wild type (wt).
- Within the E318K$^+$ cohort, R genotypes (R/R, R/r and R/wt) conferred a significantly higher risk compared with non-R genotypes (r/r, r/wt and wt/wt).
- Exome data from 200 000 UK residents consistently found that melanoma risk in *MC1R* r carriers was comparable with wt.

Internationally, melanoma is among the most common cancers in young adults generally and young women in particular.[1–3] Although population screening for melanoma is unlikely to be cost-effective,[4] targeted screening of high-risk populations may be economically feasible.[5] Recognizing those at greatest risk could facilitate targeted screening leading to early diagnosis and intervention, which are crucial to improving prognosis and outcomes.[6,7] As twin studies have estimated the heritability of melanoma to be 55–58%,[8,9] genetic information is likely to assist in risk stratification.

Variants in multiple genes confer an increased risk of melanoma. Highly penetrant mutations in familial melanoma genes, of which *Cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) is the most frequently implicated, increase lifetime risk to about 50%,[10] while moderate- and low-risk variants increase risk by approximately 2–4-fold and 1–2-fold above population level, respectively.[11,12] The two best-described moderate-risk genes are *Microphthalmia-associated transcription factor* (*MITF*) and *Melanocortin-1 receptor* (*MC1R*).[13–16] A functional variant, *MITF* E318K, has a population allele frequency of 0.6–0.8%, but it is significantly more common (1.6–2.8%) in melanoma cohorts[11,13,17] and is associated with an approximate 2.37–2.63-fold increased risk of melanoma.[18,19] *MC1R* contains nine common variants known to be predictive of red hair colour (RHC alleles), which are classified as either strong (R) or weak (r). R alleles incur a higher odds ratio (OR) of melanoma (OR 1.93) than r alleles (OR 1.55).[20,21] This risk is dose-dependent with melanoma risk increasing with each additional copy of R or r.[20]

The interaction between variants within melanoma susceptibility genes is still being investigated. However, it has been shown previously that *MC1R* variants modify the penetrance of *CDKN2A* pathogenic variants, whereby *CDKN2A* carriers who also carry RHC alleles are more likely to develop melanoma, be diagnosed at an earlier age and be diagnosed with multiple melanomas.[22–25] An interactive effect between *MC1R* RHC variants and the *MITF* E318K variant has been hypothesized previously.[17] One prior study of 97 *MITF* E318K carriers, of whom 44 had a personal history of melanoma, found no evidence that *MC1R* variants differentially modified melanoma risk in E318K carriers compared with noncarriers. However, the subset of E318K$^+$ individuals who also carried any *MC1R* RHC allele (grouping R and r) had a higher melanoma risk than E318K$^+$ individuals who were *MC1R* wild type (wt).[26] A recent study reported *MC1R* genotype frequencies in *MITF* E318K$^+$ ($n=20$) and E318K$^-$ ($n=556$) cases in a melanoma cohort, but it was not sufficiently powered to conduct analysis.[27] A functional study showed that forced expression of *MITF* leads to the development of malignant cells in *MC1R* R/R genotypes, while this does not occur in *MC1R* wt genotypes.[28] There have been no studies investigating how individual *MC1R* RHC alleles and/or genotypes moderate the risk of developing melanoma in E318K$^+$ individuals. We therefore aimed to evaluate whether *MC1R* RHC alleles and genotypes moderate melanoma risk in *MITF* E318K$^+$ and E318K$^-$ cohorts.

Materials and methods

Study design and data collection

This study used data from nine sources: (i) the Brisbane Naevi Morphology Study (BNMS),[29] a case–control study of naevus and melanoma genes in which cases had at least one histopathologically confirmed melanoma; (ii) the Australian Melanoma Family Study, a population-based case–control family study of histopathologically confirmed melanoma diagnosed under the age of 40 years;[30] (iii) histopathologically confirmed affected individuals from the University of Tübingen, Germany; (iv) the familial melanoma study at The Hospital Clinic of Barcelona,[13] in which all histopathologically confirmed melanoma-affected probands were genotyped for *MITF* E318K status and, when positive, family members were also genotyped; (v) histopathologically confirmed affected cases from Western Australian Melanoma Health Study (WAMHS), a population-based study of adult melanoma cases in Western Australia;[31] (vi) histopathologically confirmed individuals from the EPIGENE cohort;[32] and (vii) histopathologically confirmed cases and unaffected

controls from the population-based Queensland QSkin Sun and Health Study Cohort,[33] which recruited participants through the Australian Electoral Roll. Additional databases were analysed that included *MITF* E318K$^+$ individuals with and without a history of melanoma, specifically: (viii) the Medical Genome Reference Bank (MGRB), which contains genomic data on healthy elderly individuals;[34] and (ix) The Cancer Genome Atlas (TCGA), which contains genotypes on over 20 000 samples across various cancer types, including a subset with melanoma.[35] Clinical data included melanoma status (affected or unaffected), age at initial diagnosis, number of melanomas and sex. RHC frequencies in E318K$^+$ individuals with and without a history of melanoma are presented in Table S1 (see Supporting Information).

Finally, to determine whether findings in the study cohorts were reflective of the general population, interactive effects were explored in a 10th dataset, exome data from 200 000 UK residents with and without a personal history of melanoma, who were all participants in the UK Biobank study.[36] These individuals were aged between 40–69 years of age and those with a histopathologically confirmed case of melanoma were classified as affected.

Genotyping

MC1R R (D84E/rs1805006, R142H/rs11547464, R151C/rs1805007, R160W/rs1805008 and D294H/rs1805009) and r (V60L/rs1805005, V92M/rs2228479, I155T/rs1110400 and R163Q/rs885479) and the *MITF* E318K/rs149617956) variants were genotyped in the BNMS and German samples (University of Tübingen) at the University of Queensland using either Sanger sequencing or TaqMan single-nucleotide polymorphism (SNP) Genotyping Assays with polymerase chain reaction. These methods have been described previously.[17] In the Barcelona samples *MITF* E318K SNPs were genotyped using TaqMan,[13] Sanger sequencing or using gene-panel testing (Trusight Hereditary Cancer panel; Illumina, San Diego, CA, USA); and *MC1R* variants were assessed by Sanger sequencing as previously described,[37] while the Australian Melanoma Family Study samples were genotyped using Sanger sequencing.[16,20] WAMHS samples were genotyped using Illumina Infinium HumanOmniExpressExome (Illumina). EPIGENE samples were genotyped using the Illumina CoreExome array (Illumina). QSkin samples were genotyped using the Illumina Global Screening Array (Illumina). For the WAMHS, EPIGENE and QSkin cohorts, plink v1.90b6.26 was used for all steps except for converting imputed SNP dosages to hard-call genotypes which used plink v2.00a3 (14 Aug 2022).[38,39] For three *MC1R* RHC variants across three cohorts (WAMHS rs1805009, EPIGENE rs1805009 and rs1805005, and QSkin rs1805009 and rs885479), direct genotypes were not available, and data imputed to Haplotype Reference Consortium v1.1 was used (Table S2; see Supporting Information). Details of data cleaning and imputation details have been previously reported.[19]

TCGA uses microarrays to genotype or impute the genotypes for all variants. All MGRB samples underwent whole-genome sequencing.[34] The *MC1R* genotypes in E318K$^-$ individuals were obtained from cases and controls of the BNMS exclusively as the sample size was sufficiently large. It has been shown previously that frequencies of the *MC1R* variants were consistent with other affected and control cohorts.[40] In the first four cohorts, individuals were screened for high-risk variants in *CDKN2A*, and positive cases were excluded. Participants in the WAMHS, EPIGENE and QSkin cohorts were not screened for *CDKN2A* variants. *CDKN2A* sequence data were available from all TCGA samples and 13 of 19 MGRB participants and no variants were identified.

Whole-exome sequencing (WES) for the UK Biobank was performed using library preparation, exome enrichment and sequencing data processing as previously described.[41] WES data were filtered to retain good-quality variants in *CDKN2A*, which were rare (minor allele frequency < 0.05), within control populations (Genome Aggregation Database, gnomAD), were in conserved bases/regions (GERP) and/or were predicted to be deleterious using *in silico* predictions (SIFT, Polyphen2, MutationTaster, FATHMM). Any potentially deleterious variants were explored for previous disease associations (CLINVAR).

Data analysis

Logistic regression using IBM SPSS Statistics (v. 28) was used to compare melanoma ORs associated with each RHC allele and genotype in *MITF* E318K$^-$ and E318K$^+$ groups relative to the wt allele and wt/wt genotype. χ^2 analysis was used to identify any significant differences in melanoma frequencies between genotypes containing an R allele (R/R, R/r and R/wt) and genotypes not containing R allele (r/r, r/wt and wt/wt). Analyses were repeated in a population control cohort (200 000 exomes from UK Biobank).

Results

We analysed genotypic and phenotypic data from 322 E318K$^+$ heterozygotes, comprised of both melanoma-affected ($n = 136$) and unaffected ($n = 186$) individuals. The breakdown of sample origins is presented in Table 1, and the *MC1R* genotypes of *MITF* E318K$^+$ individuals from each site are shown in Table S1. The control cohort consisted of 1165 E318K$^-$ individuals, including both melanoma-affected ($n = 532$) and unaffected ($n = 633$) individuals. Specific RHC variants were reported for each cohort in Table 2.

The frequency of RHC alleles and genotypes, and associated melanoma ORs (logistic regression) in E318K$^+$ and E318K$^-$ individuals are presented in Table 3. Within the *MITF* E318K$^-$ cohort, the presence of *MC1R* RHC alleles, R and r significantly increased the OR for melanoma [OR 2.04 (95% confidence interval, CI, 1.67–2.49) and OR 1.64 (1.35–2.00), respectively] relative to the E318K$^-$ *MC1R* wt allele. Similarly, within the E318K$^-$ cohort, all RHC genotypes – R/R [OR 4.40 (2.65–7.29)], R/r [OR 3.28 (2.21–4.87)], R/wt [OR 2.19 (1.50–3.20)], r/r [OR 2.52 (1.54–4.13)] and r/wt [OR 1.91 (1.31–2.77)] – were associated with a significantly higher melanoma risk compared with the E318K$^-$ *MC1R* wt/wt genotype; $P < 0.001$ for all (Table 3).

In the E318K$^+$ cohort, the *MC1R* R allele was associated with a significantly higher risk of melanoma [OR 1.67 (1.13–2.49)] relative to the E318K$^+$ *MC1R* wt allele, while the r allele was not associated with increased melanoma risk [OR 0.78 (0.54–1.14)]. For the E318K$^+$ group, RHC genotypes – R/R [OR 2.01 (0.68–5.98)], R/r [OR 1.64 (0.77–3.49)], R/wt

Table 1 Melanoma status and source of *MITF* E318K carriers and noncarriers

				Melanoma status	
***MITF* status**	**Source**	***N***	**Recruitment**	**Affected**	**Unaffected**
***MITF* E318K⁻**	Brisbane Naevi Morphology Study	1165	Population and clinic recruitment of affected cases and unaffected family members	532	633
***MITF* E318K⁺**	Brisbane Naevi Morphology Study	25	(As above)	17	8
	QSkin	148	Histopathologically confirmed cases and unaffected controls recruited through Australian Electoral Roll	34	114
	EPIGENE	17	Histopathologically confirmed individuals recruited through a cancer registry	17	0
	WAMHS	27	Histopathologically confirmed affected cases recruited through a cancer registry	27	0
	Melanoma Unit of Hospital Clinic Barcelona	52	High-risk clinic	23	29
	German Cohort	4	Affected individuals recruited through high-risk clinic	4	0
	Australian Melanoma Family Study	24	Population-based case–control family study of melanoma diagnosed < 40 years	8	16
	The Cancer Genome Atlas	6	Genotypes from > 20 000 samples from various cancer types	6	0
	The Medical Genome Research Bank	19	4000 healthy elderly individuals	0	19

WAMHS, Western Australian Melanoma Health Study.

Table 2 Frequency of individual *MC1R* RHC variants in *MITF* E318K carriers

***MITF* E318K status**	**R alleles, *n* (%)**					**r alleles, *n* (%)**				**wt allele**
	D84E	**R142H**	**R151C**	**R160W**	**D294H**	**V60L**	**V92M**	**I155T**	**R163Q**	
MITF E318K⁺ alleles (*n* = 618)	8 (1.3)	5 (0.8)	52 (8.4)	48 (7.8)	17 (2.8)	64 (10.4)	88 (14.2)	17 (2.8)	15 (2.4)	304 (49.2)
MITF E318K⁻ alleles (*n* = 1924)	25 (1.3)	14 (0.7)	234 (12.2)	188 (9.8)	57 (3.0)	216 (11.2)	170 (8.8)	218 (11.3)	87 (4.5)	715 (37.2)
MAF (gnomAD)	0.0051	0.0051	0.0448	0.0476	0.0092	0.0842	0.0779	0.0057	0.1457	

Data were not available for specific variant data from 203 of 1165 participants from *MITF* E318K⁻ cohort. Data were not available for specific variant data from 13 of 322 participants from the *MITF* E318K⁺ cohort (three from BNMS, six from MGRB, four from the German cohort).MAF, minor allele frequency; gnomAD, Genome Aggregation Database; wt, wild type.

Table 3 Comparison of melanoma risk within each *MC1R* red hair colour allele and genotype in *MITF* E318K⁻ and E318K⁺ individuals

MC1R	***MITF* E318K⁻**			***MITF* E318K+**		
	Unaffected *n* (%)	**Melanoma *n* (%)**	**OR (95% CI)**	**Unaffected *n* (%)**	**Melanoma *n* (%)**	**OR (95% CI)**
Alleles	1266	1064		372	272	
wt	621 (49.1)	367 (34.5)	1.0	184 (49.5)	128 (47)	1.0
R	300 (23.7)	362 (34.0)	**2.04 (1.67–2.49)**	67 (18.0)	78 (28.7)	**1.67 (1.13–2.49)**
r	345 (27.3)	335 (31.5)	**1.64 (1.35–2.00)**	121 (32.5)	66 (24.3)	0.78 (0.54–1.14)
Genotypes	633	532		186	136	
wt/wt	164 (25.9)	65 (12.2)	1.0	47 (25.3)	30 (22.1)	1.0
R/R	35 (5.5)	61 (11.5)	**4.40 (2.65–7.29)**	7 (3.8)	9 (6.6)	2.01 (0.68–5.98)
R/r	93 (14.7)	121 (22.7)	**3.28 (2.21–4.87)**	21 (11.3)	22 (16.2)	1.64 (0.77–3.49)
R/wt	137 (21.6)	119 (22.4)	**2.19 (1.50–3.20)**	32 (17.2)	38 (27.9)	1.86 (0.96–3.59)
r/r	48 (7.6)	48 (9.02)	**2.52 (1.54–4.13)**	21 (11.3)	7 (5.1)	0.52 (0.20–1.38)
r/wt	156 (24.6)	118 (22.2)	**1.91 (1.31–2.77)**	58 (31.2)	30 (22.1)	0.81 (0.43–1.53)

OR, odds ratio; CI, confidence interval; **Bold** indicates statistical significance ($P < 0.05$).

[OR 1.86 (0.96–3.59)], r/r [OR 0.52 (0.20–1.38)] and r/wt [OR 0.81 (0.43–1.53)] – were not significantly associated with an increased risk of melanoma relative to the E318K⁺ *MC1R* wt/wt genotype. The r/r genotype was inversely but not significantly associated with melanoma risk [OR 0.52 (0.20–1.38), $P = 0.19$]. However, when the E318K⁺ RHC genotypes were grouped, all *MC1R* R genotypes (R/R, R/r and R/wt) were associated with significantly higher melanoma risk compared with non-R genotypes (r/r, r/wt and wt/wt) ($P < 0.001$) (data not shown in Table 3).

Evaluation of *MC1R*/*MITF* variant interactions in the UK Biobank

In 200 000 exomes from UK residents (UK Biobank) there were 1519 *MITF* E318K⁺ individuals, of whom the majority

($n = 1483$) had no documented history of melanoma and 36 had a histologically confirmed melanoma diagnosis. Within the *MITF* E318K$^-$ group (199 083) there were 1615 cases of melanoma and ORs for R and r alleles [OR 1.97 (95% CI 1.81–2.14) and OR 1.40 (1.28–1.52), respectively] were consistent with the ORs of our study cohorts (Table S3; see Supporting Information). In the E318K$^+$ group, a trend was observed for an association for a higher melanoma risk with the R allele compared with the wt allele [OR 1.65 (0.95–2.86), $P = 0.07$] and for the R/R genotype compared with the wt/wt genotype [OR 3.23 (0.89–11.72), $P = 0.06$]. Consistent with our study cohorts, the melanoma risk for r allele carriers was comparable with the wt [OR 1.12 (0.63–2.01), $P = 0.694$]. Similarly, the remaining RHC genotypes (R/r, R/wt, r/r and r/wt) were not significantly associated with the risk of melanoma relative to the wt/wt genotype. Within this E318K$^+$ cohort of the UK Biobank, we found no evidence that the risk of melanoma differed between grouped R genotypes (R/R, R/r and R/wt) and non-R genotypes (r/r, r/wt and wt/wt) ($P = 0.146$). These results should be interpreted with caution given the limited number of reported melanoma cases in the UK Biobank.

Discussion

The interaction between *MC1R* and *MITF*, two moderate-risk genes for melanoma, has long been debated in the literature.[17,26,28] Understanding the interactive relationship between *MITF* E318K and *MC1R* RHC variants may be able to help facilitate individualized risk stratification and the customization of screening recommendations. We have shown that RHC alleles and genotypes modified melanoma risk differentially in *MITF* E318K$^+$ and E318K$^-$ individuals. Specifically, the RHC R allele increased melanoma risk in E318K$^+$ and E318K$^-$ individuals, while the r allele increased risk in E318K$^-$ individuals alone.

In accordance with past research,[20,40,42] we found that in E318K$^-$ individuals, *MC1R* RHC alleles R and r were associated with an increased risk of melanoma compared with the wt. Specifically, *MC1R* R was associated with a greater risk than r allele frequencies.[20,42,43] Comparing *MC1R* genotypes across the E318K$^-$ cohort revealed that, as expected, the risk of melanoma is increased with the addition of each R and r allele, where R conferred a higher risk than r.

The risk profile differed in E318K$^+$ individuals, where only the *MC1R* R allele was significantly associated with a higher melanoma risk relative to the wt allele. The r allele was associated with the lowest OR for melanoma, which was comparable with the wt allele [OR 0.78 (95% CI 0.54–1.14) vs. 1.0, respectively]. A previous study, with a smaller cohort, grouped R and r alleles in E318K$^+$ individuals and reported that *MC1R* RHC alleles increased melanoma risk in E318K$^+$ individuals relative to *MC1R* wt alleles.[26] It is possible that the increased risk in that study was driven by the R allele alone. In our study, no RHC alleles were significantly associated with melanoma risk relative to the wt/wt genotype in E318K$^+$ individuals, although this is likely due to the small sample size. However, we did find higher melanoma frequency (approximately 2–3-fold) in genotypes containing an R allele (R/R, R/r and R/wt) compared with genotypes that did not contain an R allele. Interestingly, we also found that r/r genotypes were associated with the lowest OR for melanoma relative to the wt/wt genotype [OR 0.52 (0.20–1.38)], although again this did not reach statistical significance. A recent study in melanocyte cell lines showed that the forced expression of *MITF*, consistent with the effects of the E318K variant, leads to the development of malignant cells in *MC1R* genotype R/R cells, while this did not occur in the wt/wt genotype cells.[28] Unfortunately, the study did not explore the impact of forced expression of *MITF* on other RHC genotypes, such as r/r. Based on findings from our study, it is possible that forced expression of *MITF* may have a similar impact on cells heterozygous for the R allele and have little to no impact on cells with non-R genotypes (r/r, r/wt and wt/wt). The impact of forced expression of *MITF* on *MC1R* genotypes could possibly be related to the influence of *MC1R* RHC alleles on mutational burden. A study evaluating mutational burden in E318K$^-$ melanoma tumours relative to *MC1R* genotypes showed that R genotypes (R/R, R/r and R/wt) were associated with a significant increase in mutational burden (OR 1.68–2.86) compared with the wt/wt genotype.[44] Conversely, while the presence of one r allele (grouping R/r and r/wt genotypes) increased the mutational load (OR 1.45) compared with the wt, the r/r genotype was not associated with an increased mutational load compared with the wt (OR 0.97).[44] There is no such study in E318K$^+$ cells that could provide insight regarding whether a susceptibility to high mutational burden paired with forced expression of *MITF* is the driving factor for the development of malignant cells within this cohort.

We noted similar findings in the population-based UK Biobank cohort, although we are hesitant to overinterpret these results given the limited number of confirmed melanoma cases. Within E318K$^+$ individuals, the R allele trended ($P = 0.07$) towards being associated with a higher melanoma risk compared with the wt allele, while the r allele was comparable with the wt. In addition, no RHC genotypes were associated with increased melanoma risk relative to the wt/wt genotype.

Population-level screening for melanoma is not thought to be cost-effective in Australia as the number of people needed to screen may be too high.[4] However, targeted screening of high-risk individuals may be economically viable.[5] The information presented in this study could assist in identifying susceptible individuals, thereby facilitating targeted screening. Our findings suggest that the *MC1R* RHC R allele may increase the risk of melanoma in E318K$^+$ individuals, while the r allele does not.

To our knowledge, our study includes the largest cohort of *MITF* E318K$^+$ individuals genotyped for *MC1R* RHC alleles. However, our high-risk cohort is not sufficiently powered to detect the effects of individual *MC1R* RHC genotypes within the E318K$^+$ group. We note that there are always limitations to pooling data from different studies given nuances in recruitment and evaluation. Lack of consistency in the documentation of other important risk factors for melanoma such as age, sex, geographical location and naevi size and number meant that we were not able to account for those factors in our analysis. Furthermore, the diverse aetiology of our cohorts limited our ability to compare *MC1R* RHC frequencies in *MITF* E318K$^+$ vs. E318K$^-$ groups.

In summary, we have shown an interactive relationship between the *MITF* E318K variant and *MC1R* RHC alleles and genotypes in high-risk cohorts. Specifically, in E318K$^+$ individuals, the *MC1R* R allele increases melanoma risk relative to the wt allele while the r allele is comparable with the wt allele. If replicated in a larger cohort *MITF* and *MC1R* sequencing could further inform risk stratification and management recommendations.

Funding sources

A.M.M.-L. was funded by an National Health and Medical Research Council (NHMRC) Early Career Fellowship (ID 1158111) during this project. This work was funded by the NHMRC project grants APP1062935 and APP1083612, and the Centre of Research Excellence for the Study of Naevi APP1099021. The results published here are in whole or part based on data generated by the Medical Genome Reference Bank (MGRB) Partners (https://sgc.garvan.org.au/initiatives/mgrb). The MGRB was funded by the New South Wales State Government. The Translational Research Institute is supported by a grant from the Australian Government. The results published here are in whole or part based on data generated by The Cancer Genome Atlas (TCGA) Research Network (https://www.cancer.gov/tcga). A.E.C. receives a NHMRC Career Development Fellowship (1147843). The Australian Melanoma Family Study was supported by the NHMRC (project grants 566946, 107359 and 211172 and programme grant number 402761); the Cancer Council New South Wales (project grant 77/00, 06/10), the Cancer Council Victoria and the Cancer Council Queensland (project grant 371); and the US National Institutes of Health [NIH RO1 grant CA83115 to Genomel (www.genomel.org)]. The research at the Hospital Clínic of Barcelona is partially funded by Fondo de Investigaciones Sanitarias grants PI12/00840, PI15/00716, PI15/00956 and CM17/00042; CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain, co-financed by European Development Regional Fund 'A way to achieve Europe' ERDF; AGAUR 2014_SGR_603 of the Catalan Government, Spain; CERCA Programme/Generalitat de Catalunya; European Commission under the 6th Framework Programme, Contract No. LSHC-CT-2006-018702 (GenoMEL) and by the European Commission under the 7th Framework Programme, Diagnoptics; a grant from 'Fundació La Marató de TV3, 201331-30', Catalonia, Spain, and a grant from 'Asociación Española Contra el Cáncer (AECC)'. The EPIGENE study authors acknowledge and thank the study participants, and the support of Sullivan and Nicolaides Pathology, Queensland Medical Laboratories and IQ. EPIGENE was funded by the Australian NHMRC (APP442960). The QSkin Study recognizes and thanks the participants who make the study possible. We also wish to recognize the hard work and contributions of all staff, students and colleagues who have supported this work. Finally, we gratefully acknowledge funding from the Australian NHMRC (project grants APP106306, APP1185416 and APP1073898, and programme grant 552429). The Western Australian Melanoma Health Study was supported by The University of Western Australia and the Scott Kirkbride Melanoma Research Centre.

Conflicts of interest

H.P.S. is a shareholder of MoleMap NZ Limited and e-derm consult GmbH, and undertakes regular teledermatological reporting for both companies; he is also a Medical Consultant for Canfield Scientific Inc., Blaze Bioscience Inc., MoleMap Australia Pty Limited, and a Medical Advisor for First Derm and Revenio Research Oy. The other authors state no conflicts of interest.

Data availability

The data presented in this study are available on request from the corresponding author.

Ethics statement

Ethics approval for the Brisbane Naevi Morphology Study (BNMS) was obtained through the Metro South Human Research Ethics Committee (HREC) (HREC/09/QPAH/162) and the University of Queensland HREC (HREC: 2009001590), which covered the analysis of the Queensland and German samples. Approval for the Spanish cohort was obtained from the ethics committee at Hospital Clinic of Barcelona (ref.: 3153). Approval for the Australian Melanoma Family Study was obtained from the ethics committees at University of Sydney, University of Melbourne, University of Queensland, Cancer Council Victoria, Queensland Cancer Register and Cancer Council NSW. The QSkin study was approved by the HREC of the QIMR Berghofer Medical Research Institute (P1309). Approval for the Western Australian Melanoma Health Study (WAMHS) was obtained from the ethics committee at the University of Western Australia (refs: 2021/ET000832 and 2021/ET000486).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

References

1 American Cancer Society. *Cancer Facts & Figures* 2020. Atlanta (GA): ACC, 2020. Available at: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2020.html#:~:text=The Facts %26 Figures annual report provides%3A 1, cancer symptoms%2C risk factors%2C early detection%2C and treatment (last accessed 22 March 2023).
2 Australian Institute of Health and Welfare. *Cancer in Australia: in brief*. 2019. Available at: https://www.aihw.gov.au/reports/cancer/cancer-in-australia-2019-in-brief/summary (last accessed 22 March 2023).
3 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**:7–34. https://doi.org/10.3322/caac.21551.
4 Wernli KJ, Henrikson NB, Morrison CC *et al.* Screening for skin cancer in adults: updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA* 2016; **316**:436–47. https://doi.org/10.1001/jama.2016.5415.
5 Wilson ECF, Usher-Smith JA, Emery J *et al.* A modeling study of the cost-effectiveness of a risk-stratified surveillance program for melanoma in the United Kingdom. *Value Health* 2018; **21**:658–68. https://doi.org/10.1016/j.jval.2017.11.009.

6 Katalinic A, Waldmann A, Weinstock MA *et al.* Does skin cancer screening save lives? An observational study comparing trends in melanoma mortality in regions with and without screening. *Cancer* 2012; **118**:5395–402.
7 Schneider JS, Moore DH, Mendelsohn ML. Screening program reduced melanoma mortality at the Lawrence Livermore National Laboratory, 1984 to 1996. *J Am Acad Dermatol* 2008; **58**:741–9.
8 Shekar SN, Duffy DL, Youl P *et al.* A population-based study of Australian twins with melanoma suggests a strong genetic contribution to liability. *J Invest Dermatol* 2009; **129**:2211–19.
9 Mucci LA, Hjelmborg JB, Harris JR *et al.*; Nordic Twin Study of Cancer (NorTwinCan) Collaboration. Familial risk and heritability of cancer among twins in Nordic countries. *JAMA* 2016; **315**:68–76. https://doi.org/10.1001/jama.2015.17703.
10 Cust AE, Harland M, Makalic E *et al.* Melanoma risk for CDKN2A mutation carriers who are relatives of population-based case carriers in Australia and the UK. *J Med Genet* 2011; **48**:266–72. https://doi.org/10.1136/jmg.2010.086538.
11 Potrony M, Badenas C, Aguilera P *et al.* Update in genetic susceptibility in melanoma. *Ann Transl Med* 2015; **3**:210. https://doi.org/10.3978/j.issn.2305-5839.2015.08.11.
12 Ransohoff KJ, Jaju PD, Tang JY *et al.* Familial skin cancer syndromes increased melanoma risk. *J Am Acad Dermatol* 2016; **74**:P423–34. https://doi.org/10.1016/j.jaad.2015.09.070.
13 Potrony M, Puig-Butille JA, Aguilera P *et al.* Prevalence of MITF p.E318K in patients with melanoma independent of the presence of CDKN2A causative mutations. *JAMA Dermatol* 2016; **152**:405–12.
14 Raimondi S, Sera F, Gandini S *et al.* MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer* 2008; **122**:2753–60.
15 Bertolotto C, Lesueur F, Giuliano S *et al.* A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* 2011; **480**:94–8. https://doi.org/10.1038/nature10539.
16 Yokoyama S, Woods SL, Boyle GM *et al.* A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature* 2011; **480**:99–103. https://doi.org/10.1038/nature10630.
17 Sturm RA, Fox C, McClenahan P *et al.* Phenotypic characterization of nevus and tumor patterns in MITF E318K mutation carrier melanoma patients. *J Invest Dermatol* 2014; **134**:141–9.
18 Guhan SM, Artomov M, McCormick S *et al.* Cancer risks associated with the germline MITF(E318K) variant. *Sci Rep* 2020; **10**:17051. https://doi.org/10.1038/s41598-020-74237-z.
19 Landi MT, Bishop DT, MacGregor S *et al.* Genome-wide association meta-analyses combining multiple risk phenotypes provide insights into the genetic architecture of cutaneous melanoma susceptibility. *Nat Genet* 2020; **52**:494–504.
20 Cust AE, Goumas C, Holland EA *et al.* MC1R genotypes and risk of melanoma before age 40 years: a population-based case–control-family study. *Int J Cancer* 2012; **131**:E269-81. https://doi.org/10.1002/ijc.27357.
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**:1730–40. https://doi.org/10.1002/ijc.25804.
22 Pastorino L, Bonelli L, Ghiorzo P *et al.* CDKN2A mutations and MC1R variants in Italian patients with single or multiple primary melanoma. *Pigment Cell Melanoma Res* 2008; **21**:700–9.
23 Fargnoli MC, Gandini S, Peris K *et al.* MC1R variants increase melanoma risk in families with CDKN2A mutations: a meta-analysis. *Eur J Cancer* 2010; **46**:1413–20. https://doi.org/10.1016/j.ejca.2010.01.027.
24 Pellegrini C, Maturo MG, Martorelli C *et al.* Characterization of melanoma susceptibility genes in high-risk patients from Central Italy. *Melanoma Res* 2017; **27**:258–67. https://doi.org/10.1097/CMR.0000000000000323.
25 Box NF, Duffy DL, Chen W *et al.* MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am J Hum Genet* 2001; **69**:765–73. https://doi.org/10.1086/323412.
26 Berwick M, Macarthur J, Orlow I *et al.* MITF E318K's effect on melanoma risk independent of, but modified by, other risk factors. *Pigment Cell Melanoma Res* 2014; **27**:485–8.
27 Ciccarese G, Dalmasso B, Bruno W *et al.*; Italian Melanoma Intergroup (I.M.I.). Clinical, pathological and dermoscopic phenotype of MITF p.E318K carrier cutaneous melanoma patients. *J Transl Med* 2020; **18**:78. https://doi.org/10.1186/s12967-020-02253-8.
28 Lavelle TJ, Alver TN, Heintz KM *et al.* Dysregulation of MITF leads to transformation in MC1R-defective melanocytes. *Cancers (Basel)* 2020; **12**:1719. https://doi.org/10.3390/cancers12071719.
29 McWhirter SR, Duffy DL, Lee KJ *et al.* Classifying dermoscopic patterns of naevi in a case-control study of melanoma. *PLoS One* 2017; **12**:e0186647. https://doi.org/10.1371/journal.pone.0186647.
30 Cust AE, Schmid H, Maskiell JA *et al.* Population-based, case-control-family design to investigate genetic and environmental influences on melanoma risk. *Am J Epidemiol* 2009; **170**:1541–54.
31 Ward SV, Cadby G, Lee A *et al.* The Western Australian Melanoma Health Study: study design and participant characteristics. *Cancer Epidemiol* 2011; **35**:423–31. https://doi.org/10.1016/j.canep.2011.02.010.
32 Kvaskoff M, Pandeya N, Green AC *et al.* Site-specific determinants of cutaneous melanoma: a case-case comparison of patients with tumors arising on the head or trunk. *Cancer Epidemiol Biomarkers Prev* 2013; **22**:2222–31. https://doi.org/10.1158/1055-9965.EPI-13-0475.
33 Olsen CM, Green AC, Neale RE *et al.* Cohort profile: the QSkin Sun and Health Study. *Int J Epidemiol* 2012; **41**:929–9i. https://doi.org/10.1093/ije/dys107.
34 Pinese M, Lacaze P, Rath EM *et al.* The Medical Genome Reference Bank contains whole genome and phenotype data of 2570 healthy elderly. *Nat Commun* 2020; **11**:435. https://doi.org/10.1038/s41467-019-14079-0.
35 Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)* 2015; **19**:A68–77. https://doi.org/10.5114/wo.2014.47136.
36 Bycroft C, Freeman C, Petkova D *et al.* The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018; **562**:203–9. https://doi.org/10.1038/s41586-018-0579-z.
37 Vallone MG, Tell-Marti G, Potrony M *et al.* Melanocortin 1 receptor (MC1R) polymorphisms' influence on size and dermoscopic features of nevi. *Pigment Cell Melanoma Res* 2018; **31**:39–50.
38 Chang CC, Chow CC, Tellier LC *et al.* Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015; **4**:7. https://doi.org/10.1186/s13742-015-0047-8.
39 Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**:559–75. https://doi.org/10.1086/519795.
40 Duffy DL, Jagirdar K, Lee KJ *et al.* Genes determining nevus count and dermoscopic appearance in Australian melanoma cases and controls. *J Invest Dermatol* 2020; **140**:498–501.e17.
41 Rayner JE, McMeniman EK, Duffy DL *et al.* Phenotypic and genotypic analysis of amelanotic and hypomelanotic melanoma patients. *J Eur Acad Dermatology Venereol* 2019; **33**:1076–83. https://doi.org/10.1111/jdv.15446.
42 Vernali S, Waxweiler WT, Dillon PM *et al.* Association of incident amelanotic melanoma with phenotypic characteristics, MC1R status, and prior amelanotic melanoma. *JAMA Dermatol* 2017; **153**:1026–31.
43 Duffy DL, Lee KJ, Jagirdar K *et al.* High naevus count and MC1R red hair alleles contribute synergistically to increased melanoma risk. *Br J Dermatol* 2019; **181**:1009–16.
44 Johansson PA, Pritchard AL, Patch AM *et al.* Mutation load in melanoma is affected by MC1R genotype. *Pigment Cell Melanoma Res* 2017; **30**:255–8.