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MC1R variant alleles and malignant melanoma risk in Israel

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ABSTRACT

To evaluate the contribution of *MC1R* variants to malignant melanoma risk in Israeli Jews, sequencing of the *MC1R* gene was performed in 132 melanoma patients and 184 ethnically matched controls. Overall, 22 *MC1R* variants were detected, two were novel (M73I and 496_497insG). Using age and sex-adjusted logistic regression, one specific variant, R151C, conferred significantly increased melanoma risk among Ashkenazim (OR = 2.6, 95% CI: 1.3–5.3; $p = 0.05$ after Bonferroni correction). A gene dosage effect was noted, with significantly increased melanoma risk being observed in subjects with at least two variants whether when all variants are pooled (OR = 4.8, 95% CI: 2.0–11.2; $p = 0.002$ after Bonferroni correction) or when red hair colour (RHC) variants and non-RHC variants are distinguished (OR = 7.6, 95% CI: 2.8–20.3; $p = 0.0004$ after Bonferroni correction). If further studies support these findings, the assessment of *MC1R* status may be useful in identifying Jewish Israeli individuals at high risk for melanoma.

1. Introduction

In Israel, like in other world populations,[1] the incidence of malignant melanoma (MM) is increasing: ~3-fold increase among the Jewish population, from 1970 to 1995, with ~1200 new MM cases reported annually to the Israeli National Cancer Registry in 2004–2005.[2] MM rates in Israel vary by ethnicity, with high incidence rates among Ashkenazi Jews, who typically display fair skin and hair phenotype [age standardised rates (ASRs) of Israeli-born (33.08 and 20.98/100,000 for males and females, respectively) and European–American born (14.14 and 14.69/100,000 for males and females, respectively)], compared with non-Ashkenazi (Sephardic) Jews.[2] The latter are Jews originating from Middle East and Asian countries, North Africa and Yemen, who typically display a darker phenotype [age standardised rates of MM among Asians (5.57 and 2.68/100,000 for males and females, respectively) and African born (7.05 and 6.42/100,000 for males and females, respectively)].[2]

While the majority of MM cases are sporadic, familial clustering is noted in about 10% of incident cases.[3] Germline mutations in the *CDKN2A/ARF* gene[4] were reported in 25–50% of ethnically diverse MM families with at least three melanoma cases.[3–5] In a handful of MM families worldwide, germline mutations in the *CDK4* protooncogene were reported.[6–8] The contribution of *CDKN2A/ARF* and *CDK4* germline mutations to inherited predisposition to MM in Jewish Israeli families is limited: *CDKN2A/ARF* mutations were

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reported in 4/147 families (2.7%), whereas none exhibited a *CDK4* mutation.[9–11] These data suggest that other genes underlie inherited predisposition to MM in Jewish Israeli individuals.

In addition to these two high-penetrance genes, low-risk melanoma genes have been characterised by candidate gene studies[3] and more recently by genome-wide association studies.[12,13] Most studies on candidate genes led to inconsistent results, except for the melanocortin-1 receptor gene (*MC1R*; MIM# 155555),[14] a major determinant of human pigmentation.[15] The human *MC1R* encodes for a seven-pass transmembrane, G-protein coupled-receptor of 317 amino acids.[16] The ligand, alpha melanocyte stimulating hormone (α-MSH), binds to *MC1R* with resultant production of a mixture of the photo-protective eumelanin and the photosensitiser phaeomelanin.[17]

The *MC1R* gene is highly polymorphic, with over 70 non-synonymous variant alleles reported world wide.[18] Several MC1R missense mutations (e.g. D84E, R151C, R160 W, D294H), collectively referred to as 'red hair colour' (RHC) variants, have been associated with red hair, fair skin, freckles and poor tanning ability – all known MM risk factors.[14,19] Furthermore, some *MC1R* variants have been reported in ethnically diverse populations to increase MM risk[20–24] and non-melanoma skin cancer risk,[25] above and beyond its associated effect on the pigmentary phenotype.

In this case-control study, we evaluated the putative contribution of *MC1R* variants to MM risk in the Jewish Israeli population by genotyping of the *MC1R* coding region.

2. Materials and methods

2.1. MM patients' recruitment

All patients with histopathologically confirmed MM who attended the Pigmented Lesion Dermatological Clinic at the Sheba Medical Centre from 1 January 2001 to 31 August 2006 were eligible. Melanoma patients were subdivided into three categories: (1) 'Familial cases': MM patients from families with at least two first- or second-degree relatives with MM from the same side of the pedigree. Only one MM patient was genotyped in these families. (2) 'Multiple primary melanoma (MPM) cases': patients with at least two primary MMs. This category was further subdivided into patients with or without family history of MM. (3) 'Sporadic cases': patients with a single primary MM, without family history of MM. The study was approved by both the Ethics Committees of the Sheba Medical Centre, and the Israeli Ministry of Health. All the participants signed a written informed consent. Data were acquired by personal interviews using a detailed questionnaire pertaining to demographic characteristics, ethnicity, personal sun exposure habits, sun sensitivity and history of personal and familial cancers. A dermatological examination assessing skin phenotype and pigmentation characteristics was performed by an experienced dermatologist. Confirmation of MM diagnosis was based on pathology reports, patients' medical charts, operation reports, death certificate and/or archives of the Israel Cancer Registry.

2.2. Control samples recruitment

Age (±4 years), sex and ethnically matched controls were recruited from a pool of unrelated healthy individuals. The control samples were obtained from the Oncogenetics Unit in the Sheba Medical Centre. These controls were individuals who escorted the counselled high risk individual during the oncogenetic counselling visit, and were genetically unrelated to him/her (e.g. spouse and friends). Specifically, eligible individuals had no personal or family history of MM or non-melanoma skin cancer. All control subjects agreed to participate in the study and signed a written informed consent.

2.3. DNA extraction

Ten millilitres of anticoagulated venous blood were withdrawn for genomic DNA extraction from leucocytes using the PUREGene DNA extraction kit (Gentra Inc., Minneapolis, MN), according to the manufacturer's protocol.

2.4. MC1R genotyping

The entire *MC1R* coding sequence was amplified by two PCRs, resulting in two fragments (502 bp and 618 bp) with an overlap of 101 bp. PCRs were preformed in a final volume of 50 μl containing 50–100 ng genomic DNA, PCR buffer (Fisher Biotec, West Perth, Australia), 2.5 mM $MgCl_2$, 200 nM dNTPs, 10 pmol of each primer and 0.2 U *FB1* DNA Polymerase (Fisher Biotec). Amplification was carried out as follows: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles at 94 °C for 20 s, annealing at 51 °C and 59 °C for 1 min (for the first and second fragments, respectively), extension at 72 °C for 20 s, and a final extension step at 72 °C for 5 min. Primer sequences for the detection of *MC1R* variants have been reported in previous studies.[21]

2.5. Sequence analysis

Bi-directional sequencing was carried out using the BIG DYE technology, fluorescent dyes and running the PCR products on an ABI Prism 3100 semi automatic DNA sequencer (PE Biosystems, Foster City, CA). Sequences of the *MC1R* coding region were manually determined and compared with the wild type sequence of the *MC1R* gene (GenBank accession number AF326275).

2.6. Statistical analysis

Comparisons of demographic characteristics and *MC1R* allele frequencies between groups of subjects defined by ethnic origin (Ashkenazi/non-Ashkenazi subjects) and/or disease status (cases/controls) were made by a Fisher's exact test or a Pearson's χ^2 test, depending on sample size. For each *MC1R* variant, departure of genotype frequencies from Hardy–Weinberg equilibrium was tested by a χ^2 test in controls. Association between MM risk and non-synonymous *MC1R* variants was assessed using unconditional logistic regression analysis. This analysis was adjusted for the effects of age and sex. The measure of association between melanoma risk and *MC1R* variants was the odds-ratio (OR), with its 95% confidence

interval (CI). We first evaluated the most frequent *MC1R* variants (allele frequency ⩾ 1%) individually, by comparing, for each variant, carriers of at least one variant (homozygotes and heterozygotes pooled) to subjects that did not carry that variant (reference category). Because many *MC1R* variants were too rare to examine their individual associations with MM risk, we have focused on the following *MC1R* variables: carriers of any *MC1R* variant, compared to homozygotes for the *MC1R* consensus sequence; carriers of multiple (1, ⩾2) variants, compared to consensus homozygotes [number of variants]; carriers of ⩾1 RHC variant (and no non-RHC (NRHC) variant), ⩾1 NRHC variant (and no RHC variant), or ⩾1 RHC and ⩾1 NRHC variants, compared to homozygotes for *MC1R* consensus sequence [number and types of variants]. RHC variants included D84E, R151C, R160W D294H, while all other non-synonymous variants were classified as NRHC. These analyses were repeated by examining separately the subgroup of cases with family history. We also compared the median age at diagnosis of initial melanoma first among the three group of cases (familial cases, cases with multiple primaries and sporadic cases) and then according to the presence of any *MC1R* variant, RHC and NRHC variants, as well as the number of *MC1R* variants, using the non-parametric Jonckheere–Terpstra test. All analyses were performed using the SAS software (9.1) (SAS software, Cary, NC, USA).

3. Results

3.1. Descriptive characteristics

Overall, 316 subjects participated in the study: 132 cases and 184 controls. Among the 132 MM cases 50 were familial, 30 with MPM, 5 were MPM cases with family history and 47 were sporadic. The descriptive characteristics of the study participants are presented in Table 1. Cases and controls did not differ by ethnic origin (Ashkenazim and non-Ashkenazim), the great majority (>80%) of cases or controls being Ashkenazim. Four MM cases of mixed origin were excluded from all analyses and are not included in Table 1. The proportion of males was significantly higher among MM patients (46.9%) than among controls (35.3%) ($p = 0.04$). The median age at melanoma diagnosis (25–75 quartiles) was 49 years (38–59) and in controls, the median age at blood drawing was 46 years (34–56), a statistically insignificant difference ($p = 0.08$). The distribution of the four categories of melanoma (i.e. familial, MPM with and without family history, and sporadic melanoma) were similar in Ashkenazim and non-Ashkenazim ($p = 0.58$) (Table 2).

3.2. MC1R variant determination

Table 3 displays *MC1R* variants and their observed allele frequencies by disease status and subjects' ethnic origin. Overall, 22 *MC1R* sequence variants were detected; 18 corresponded to non-synonymous amino acid substitutions, three resulted in synonymous amino acid changes and one was an insertion. Two of these *MC1R* variants (i.e. M73I and 496_497insG) are novel and have not been previously reported. One of these novel variants (496_497insG) as well as another variant (Y152X) led to a predicted truncated inactivated protein. No variant showed significant departure from Hardy–Weinberg equilibrium.

We first compared allele frequencies of each *MC1R* variant between Askhenazi and non-Askhenazi subjects in cases and controls separately. Altogether, three rare variants (R213W, R306H and Q233Q) were not observed in Ashkenazim, while nine variants (F45L, S83P, D84E, V122M, R151C, P256S, D294H, p.496_497insG and A103A) were not observed in non-Ashkenazim. When considering individual variants observed at least once in Ashkenazim and non-Ashkenazim, no significant difference in allele frequencies was detected between these two groups in either cases or controls.

Table 1 – Characteristics of malignant melanoma (MM) cases and controls.

Characteristics	MM cases ($N = 128$) (%)	Controls ($N = 184$) (%)	p-Value
Ethnic origin (n, %)			
Ashkenazim	110 (85.9)	162 (88.0)	0.58
Non-Ashkenazim	18 (14.1)	22 (12.0)	
Sex, no males (%)	60 (46.9)	65 (35.3)	0.04
Age[a] (years), median (25–75% quartiles)	49 (38–59)	46 (34–56)	0.08

a Age is the age at melanoma diagnosis in cases and age at blood drawing in controls.

Table 2 – Distribution of melanoma categories in Ashkenazi and non-Ashkenazi patients.

MM category	Ashkenazi patients ($N = 110$) (%)	Non-Ashkenazi patients ($N = 18$) (%)
Familial melanoma[a]	43 (39.1)	5 (27.8)
MPM[b]	23 (20.9)	6 (33.3)
MPM[b] with family history	4 (3.6)	1 (5.6)
Sporadic melanoma	40 (36.4)	6 (33.3)

a Familial melanoma = MM patients from families with at least two first- or second-degree relatives with MM from the same side of the pedigree.
b MPM = multiple primary melanoma.

When *MC1R* variants were pooled, we found that the distribution of the number of non-synonymous *MC1R* variants did not differ between Ashkenazi and non-Ashkenazi cases ($p = 0.13$), while it did differ significantly in controls ($p < 0.001$): 77.8% of Ashkenazi controls had at least one non-synonymous variant while that proportion was 50% in non-Ashkenazi controls. Differences between these two control groups were also observed for both RHC and NRHC variants. No RHC variant was observed in non-Ashkenazi controls, compared to a frequency of 30.8% in Ashkenazi controls, while the frequency of NRHC variants was 50% in non-Ashkenazim and 72.3% in Ashkenazim ($p = 0.003$). Because of these differences between Ashkenazi and non-Ashkenazi controls and the limited number of non-Ashkenazi participants, all subsequent analyses were confined to Ashkenazim.

3.3. *Effect of MC1R variants on MM risk in Ashkenazi subjects*

Table 4 presents the ORs between MM risk and *MC1R* variants in Askhenazi subjects. When the eight most frequent variants were examined individually, two variants, R151C and I155T, led to a significant increase in MM risk (OR = 2.6, 95% CI: 1.3–5.3 for R151C and OR = 6.8, 95% CI: 1.4–33.3 for I155T) but only the effect of R151C remained significant after Bonferroni correction ($p = 0.05$). All other variants were not significantly associated with MM, although all of them except R163Q had ORs greater than 1.0. As compared to the homozygous consensus sequence, carriage of any non-synonymous *MC1R* variant was significantly associated with MM risk (OR = 3.6, 95% CI: 1.6–8.1, $p = 0.01$ after Bonferroni correction). There was an increase in MM risk with increasing number of variants. The

Table 3 – MC1R allele frequencies in melanoma cases and controls according to ethnic origin (Ashkenazim and non-Ashkenazim).

Nucleotide change	Amino acid change	Controls		Cases	
		Ashkenazim (no. chromosomes[a] = 324) N[b] (%)	Non-Ashkenazim (no. chromosomes[a] = 44) N[b] (%)	Ashkenazim (no. chromosomes[a] = 220) N[b] (%)	Non-Ashkenazim (no. chromosomes[a] = 36) N[b] (%)
Consensus	None	133 (41.0)	31 (70.5)	50 (22.7)	10 (27.8)
Non-synonymous					
g.133T>C	F45L	0 (0)	0 (0)	1 (0.5)	0 (0)
g.178T>G	V60L	96 (29.6)	9 (20.5)	65 (29.5)	10 (27.8)
g.219G>A	M73I	1 (0.3)	0 (0)	0 (0)	1 (2.8)
g.247T>C	S83P	2 (0.6)	0 (0)	5 (2.3)	0 (0)
g.252C>A	D84E	2 (0.6)	0 (0)	1 (0.5)	0 (0)
g.274G>A	V92M	20 (6.2)	0 (0)	16 (7.3)	1 (2.8)
g.364G>A	V122M	3 (0.9)	0 (0)	2 (0.9)	0 (0)
g.425G>A	R142H	5 (1.5)	2 (4.5)	10 (4.5)	3 (8.3)
g.451C>T	R151C	16 (4.9)	0 (0)	26 (11.8)	0 (0)
g.456C>A	Y152X	2 (0.6)	0 (0)	2 (0.9)	1 (2.8)
g.464T>C	I155T	2 (0.6)	1 (2.3)	8 (3.6)	0 (0)
g.478C>T	R160W	14 (4.3)	0 (0)	14 (6.4)	4 (11.1)
g.488G>A	R163Q	16 (4.9)	0 (0)	8 (3.6)	2 (5.6)
g.637C>T	R213W	0 (0)	0 (0)	0 (0)	1 (2.8)
g.766C>T	P256S	1 (0.3)	0 (0)	0 (0)	0 (0)
g.861C>G	I287M	1 (0.3)	0 (0)	2 (0.9)	1 (2.8)
g.880G>C	D294H	0 (0)	0 (0)	1 (0.5)	0 (0)
g.917G>A	R306H	0 (0)	0 (0)	0 (0)	1 (2.8)
Insertion					
g.496_497insG		0 (0)	0 (0)	1 (0.5)	0 (0)
Synonymous					
g.309C>T	A103A	0 (0)	0 (0)	1 (0.5)	0 (0)
g.699G>A	Q233Q	0 (0)	0 (0)	0 (0)	1 (2.8)
g.942A>G	T314T	24 (7.4)	1 (2.3)	25 (11.4)	1 (2.8)
Any non-synonymous or insertion variant					
0		72 (22.2)	22 (50.0)	16 (7.3)	6 (16.7)
1		142 (43.8)	20 (45.5)	84 (38.2)	10 (27.8)
⩾2		110 (34.0)	2 (4.5)	120 (54.5)	20 (55.5)
Any variant					
0		68 (21.0)	22 (50.0)	16 (7.3)	4 (11.1)
1		130 (40.1)	18 (40.9)	68 (30.9)	12 (33.3)
⩾2		126 (38.9)	4 (9.1)	136 (61.8)	20 (55.6)

a Total number of chromosomes sequenced.
b Number of chromosomes containing the studied variant.

Table 4 – Effect of specific MC1R variants, number and type of variants on melanoma risk in Ashkenazi subjects.

MC1R variants	Cases (*n*)	Controls (*n*)	OR[a]	95% Confidence interval (CI)	*p*-Value[c]
Most frequent variants[b]					
V60L					
VV	50	84	1.0	Reference	
VL/LL	60	78	1.3	0.8–2.1	0.33
S83P					
SS	105	160	1.0	Reference	
SP/PP	5	2	4	0.7–21.1	0.10
V92M					
VV	95	143	1.0	Reference	
VM/MM	15	19	1.2	0.6–2.4	0.70
R142H					
RR	101	157	1.0	Reference	
RH/HH	9	5	2.7	0.9–8.3	0.09
R151C					
RR	86	146	1.0	Reference	
RC/CC	24	16	2.6	1.3–5.3	0.006 (0.05)
I155T					
II	102	160	1.0	Reference	
IT/TT	8	2	6.8	1.4–33.3	0.024 (0.19)
R160W					
RR	97	148	1.0	Reference	
RW/WW	13	14	1.4	0.6–3.1	0.40
R163Q					
RR	102	147	1.0	Reference	
RQ/QQ	8	15	0.8	0.3–2.0	0.60
Pooled variants					
Any variant					
No	8	36	1.0	Reference	
Yes	102	126	3.6	1.6–8.1	0.002 (0.01)
Number of variants					
0	8	36	1.0	Reference	
1	42	71	2.6	1.1–6.2	0.03 (0.18)
⩾2	60	55	4.8	2.0–11.2	0.0003 (0.002)
Number and type of *MC1R* variants					
0	8	36	1.0	Reference	
⩾1 RHC	11	16	3.0	1.0–8.9	0.05 (0.30)
⩾1 NRHC	64	94	3.0	1.3–6.9	0.01 (0.06)
⩾1 RHC and ⩾1 NRHC	27	16	7.6	2.8–20.3	0.00006 (0.0004)

a Odds-ratio (OR) is adjusted for age and sex.
b *MC1R* variants with a frequency higher than or equal to 1%.
c *p*-Values corresponding to Wald-tests; for all nominal *p*-values less than the 5% level, *p*-values after Bonferroni correction for the number of independent tests are shown in parentheses. We considered separately tests of association for specific variants and tests for pooled variants.

MM risk was ~2.0-fold higher in carriers of at least two *MC1R* variants (OR = 4.8, 95% CI: 2.0–11.2) than in those carrying only one *MC1R* variant (OR = 2.6, 95% CI: 1.1–6.2). When considering both number and type of variants, carrying both RHC and NRHC variants led to a significant increase in MM risk (OR = 7.6, 95% CI: 2.8–20.3), that was ~2.5-fold higher than the risk associated with carrying of either at least one RHC variant but no NRHC variant (OR = 3.0, 95% CI: 1.0–8.9), or at least one NRHC variant, but no RHC variant (OR = 3.0, 95% CI: 1.3–6.9). Carriage of at least two variants or at least one RHC and one NRHC variant remained significant after Bonferroni correction (Table 4).

Analyses of the subset of cases with family history led to similar results as in the whole sample (Table 5), with a significantly increased risk observed with R151C variant ($p = 0.008$ after Bonferroni correction) and carriage of at least two variants ($p = 0.018$ for having at least one RHC and one NRHC variant after Bonferroni correction).

3.4. *Effect of MC1R variants on the age at the diagnosis of MM in Ashkenazi patients*

We first checked that the median age diagnosis did not differ significantly between familial cases (46 years), cases with multiple primaries (47 years) and sporadic cases (53.5 years; $p = 0.06$). Although the median age at melanoma diagnosis in carriers of at least one *MC1R* variant (49 years) was lower

Table 5 – Effect of specific MC1R variants, number and type of variants on familial melanoma risk in Ashkenazi subjects.

MC1R variants	Familial cases (*n*)	Controls (*n*)	OR[a]	95% Confidence interval (CI)	*p*-Value[c]
Most frequent variants[b]					
V60L					
VV	25	84	1.0	Reference	
VL/LL	22	78	0.9	0.5–1.8	0.87
S83P					
SS	46	160	1.0	Reference	
SP/PP	1	2	1.7	0.2–19.6	0.65
V92M					
VV	41	143	1	Reference	
VM/MM	6	19	1.1	0.4–3.0	0.84
R142H					
RR	44	157	1	Reference	
RH/HH	3	5	2.2	0.5–9.4	0.31
R151C					
RR	33	146	1	Reference	
RC/CC	14	16	3.9	1.7–8.8	0.001 (0.008)
I155T					
II	43	160	1	Reference	
IT/TT	4	2	7.4	1.3–42.0	0.02 (0.16)
R160W					
RR	43	148	1	Reference	
RW/WW	4	14	1.0	0.3–3.1	0.98
R163Q					
RR	42	147	1	Reference	
RQ/QQ	5	15	1.2	0.4–3.4	0.78
Pooled variants					
Any variant					
No	4	36	1	Reference	
Yes	43	126	3.1	1.0–9.2	0.04 (0.24)
Number of variants					
0	4	36	1	Reference	
1	17	71	2.2	0.7–6.9	0.19
$\geqslant 2$	26	55	4.3	1.4–13.3	0.02 (0.12)
Number and type of *MC1R* variants					
0	4	36	1	Reference	
$\geqslant 1$ RHC	7	16	4.0	1.0–15.7	0.05 (0.3)
$\geqslant 1$ NRHC	24	94	2.3	0.7–7.1	0.15
$\geqslant 1$ RHC and $\geqslant 1$ NRHC	12	16	6.8	1.9–24.2	0.003 (0.018)

a Odds-ratio (OR) is adjusted for age and sex.
b *MC1R* variants with a frequency higher than or equal to 1%.
c *p*-Values corresponding to Wald-tests; for all nominal *p*-values less than the 5% level, *p*-values after Bonferroni correction for the number of independent tests are shown in parentheses. We considered separately tests of association for specific variants and tests for pooled variants.

than in non-carriers (58 years), this difference was not statistically significant ($p = 0.10$). Moreover, the median age at diagnosis was similar in patients carrying one variant (49 years) and in those having at least two variants (48.5 years). Similar results were noted when analysis was performed separately for RHC variants and NRHC variants ($p = 0.08$ and $p = 0.13$, respectively).

4. Discussion

The *MC1R* gene is highly polymorphic, mainly among Caucasian populations.[26] In this study, 22 *MC1R* variants were identified in Israeli Jewish individuals, two of which (i.e. M73I and 496_497insG) are novel. The 496_497insG variant leads to premature termination of the *MC1R* protein. The male patient who was found to be heterozygous for this variant is an Ashkenazi sporadic MM case, diagnosed at the age of 56 years, which is higher than the median age of diagnosis reported in the current study (49 years). Though no conclusion can be made based on a single case, it seems that although this variant presumably leads to a non-functional protein, it has little effect on MM phenotype.

The Ashkenazi study group displays significantly higher frequency of pooled non-synonymous variants in controls,

compared with their non-Ashkenazi counterparts. This was also true for both RHC and NRHC variants, with not even a single RHC variant noted in non-Ashkenazi controls. Among RHC variants, R151C and R160W were the most frequent in Ashkenazim, and are known to be associated with fair skin and poor tanning ability,[20,25] phenotypic features commonly observed among Ashkenazi Jews. Frequencies of these variants in the Ashkenazi controls are intermediate between those reported in Mediterranean and North-European populations, being, respectively, lowest in Greece and highest in Britain and Ireland.[27] As in several other Caucasian populations, the most frequent variant in Ashkenazi controls was V60L. The V60L frequency of 29.6% in Ashkenazim is the highest observed to date, compared to other populations.[27] Recent functional studies have revealed *MC1R* impairment conferred by this variant,[28] but V60L is more often associated with fair/blonde hair, than with the RHC phenotype or MM.[29] It should also be noted that the overall frequency of non-synonymous variants in controls (77.8% in Ashkenazim and 50% in non-Ashkenazim) is much higher than in European populations (ranging from 21.3% in Greece to 57.0% in Britain/Ireland)[27] and may account in part for the higher incidence of MM in Israel, compared to other countries.

Among the eight most frequent *MC1R* variants, R151C increased significantly MM risk among Ashkenazim. This variant was reportedly associated with the highest ORs for MM risk in a recent meta-analysis of European ancestry populations.[29] Furthermore, the association of the R151C variant with MM and its functional impairment of the *MC1R* protein are well documented.[30] The non-significant association of the other specific variants with melanoma may partly be due to the low relative risk that they confer, as observed in other populations,[29] or due to the high proportion of both cases and controls carrying at least one variant at any locus.

A gene dosage effect of *MC1R* variants was observed with a significant increase in MM risk, depending on the number and type of variants. Carrying both RHC and NRHC variants conferred the highest risk; 7.6-fold increase in risk compared to non-carriers of *MC1R* variants and a 2.5-fold increase in risk compared to subjects carrying one or more variants of either type. Similar results were noted for the subset of cases with family history. All these results are in agreement with what has been reported in other populations.[22–24,29]

In the present study, Ashkenazi carriers of *MC1R* variants had a lower median age at diagnosis (49 years) compared to non-carriers (58 years). The lack of significance of that difference may be at least partly due to the small number of non-carrier cases. The association between *MC1R* variants and median age at diagnosis was assessed in previous case-control studies of French[22] and Greek[24] populations. While in the Greek study, there was no difference in age at diagnosis, the French study reported an older age at diagnosis in *MC1R* variant carriers than in non-carriers, though this difference was not statistically significant. These inconsistent results may stem from small sample sizes; a larger study population is warranted to further analyse the effect of *MC1R* variants on age at diagnosis of MM. A larger sample size would also allow investigating this effect according to the number of melanomas, since a reduction in age at melanoma diagnosis with the number of *MC1R* variants was observed in multiple primary melanomas but not in single primary melanomas in MM prone families with *CDKN2A* mutations.[31]

A major limitation in this study is the lack of information on the prevalence of the known phenotypic MM risk factors such as hair, skin and eye colour, history of acute sunburn episodes, tanning ability, freckles density and naevus count in the control group. Hence, the analysis of association of *MC1R* variants with MM risk did not take into account these phenotypic characteristics. While the fact that ~40% of cases analysed herein represent familial cases limits our ability to draw conclusions regarding the role of *MC1R* sequence variants in sporadic melanoma cases, such enrichment may in fact serve to strengthen the power of the present study to elucidate the role of these variants in familial cases. Further studies are warranted to evaluate in the Jewish population whether *MC1R* may increase MM risk above and beyond its documented effects on pigmentation characteristics. Another limitation is the lack of information on the *CDKN2A* mutation status in the controls, but since *CDKN2A* mutations are rare among Jewish MM patients,[9–11] we assume that ignoring *CDKN2A* mutation status did not affect our findings.

In conclusion, we report for the first time that *MC1R* variants are significantly more prevalent among Ashkenazi than non-Ashkenazi Israeli Jews, accounting – at least in part – for the higher MM risk observed among Ashkenazi Jews. Furthermore, Israeli Ashkenazi Jews that carry the R151C *MC1R* variant or at least two *MC1R* variants are at increased risk to develop MM. If these data are confirmed in a larger series, it would suggest that *MC1R* status might be informative above and beyond Jewish ethnicity for more accurate identification of high risk individuals in Israel to be targeted for early detection and prevention of MM.

Conflict of interest statement

None declared.

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