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BRIEF COMMUNICATION

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Germline *MC1R* variants and frequency of somatic *BRAF*, *NRAS*, and *TERT* mutations in melanoma: Literature review and meta-analysis

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Abstract

Germline variants of the melanocortin-1-receptor (*MC1R*) gene are the most common genetic trait predisposing to cutaneous melanoma (CM). Here, we performed a literature review and meta-analysis of the association between *MC1R* gene variants and the frequency of somatic mutations of the *BRAF*, *NRAS*, and *TERT* genes in CM patients. We included studies published until January 2020 in MEDLINE, EMBASE, Ovid Medline, and two grey literature databases. Random effect models were used to pool study-specific estimates into summary odds ratio (SOR) and 95% confidence intervals (CIs). Subgroup and sensitivity analyses were conducted to identify potential sources of heterogeneity and assess the robustness of pooled estimates. Twelve studies published between 2006 and 2018 (encompassing 3566 CM, mostly on nonacral sites) were included. *MC1R* gene variants were not significantly associated with the frequency of somatic mutations of the *BRAF* and *NRAS* genes. Only three studies focused on somatic mutations of the *TERT* gene promoter, all of which reported moderate-to-strong positive associations with *MC1R* germline variants. *MC1R* gene variants appear to make only moderate changes, if any, to the risk of *BRAF*- or *NRAS*-mutant CM. The association with *TERT* promoter mutations is suggestive, yet it warrants confirmation as it is based on a still limited number of studies

KEYWORDS
germline variants, *MC1R*, melanoma, meta-analysis, somatic mutations

1 | INTRODUCTION

Cutaneous melanoma (CM) pathogenesis depends upon the interplay between environmental factors (e.g., exposure to ultraviolet [UV] radiation) and genetic predisposition. Germline variants of the melanocortin-1-receptor (*MC1R*) gene are the most widespread genetic trait predisposing to CM.[1,2] Located on chromosome 16q24.3, *MC1R* encodes a G protein-coupled receptor for the melanocyte-stimulating hormone. Upon ligand binding, MC1R activates a cyclic-adenosine monophosphate (AMP) signaling pathway leading to enhanced production of the dark, efficiently photoprotective eumelanin pigment.[3,4] *MC1R* is a highly polymorphic gene with over 200 variants identified, some of which affect eumelanin synthesis and result in increased synthesis of the red, less

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photoprotective pheomelanin. Depending on the strength of their association with the red hair phenotype, *MC1R* variants are labeled as "R" (high risk) and "r" (low risk).[5] Of note, while lighter skin and hair color are important phenotypic risk factors for CM,[6] the effect of *MC1R* variants on CM risk is partly mediated through phenotype-independent mechanisms.[7]

CM typically exhibits a high mutational burden.[8,9] Hotspot mutations in the *BRAF* and *NRAS* genes are the most common genetic alterations in CM and lead to activation of the mitogen-activated protein kinase (MAPK) pathway and loss of cell cycle regulation.[10] UV light-induced C > T transitions (known as "UV signature" mutations) of the telomerase reverse transcriptase (*TERT*) gene promoter are also common and early events in CM development.[11] *MC1R* variants are a major determinant of one's phenotype and also dysregulate pathways not involved in skin pigmentation, and several authors studied whether mutations at the *BRAF*, *NRAS*, and *TERT* genes are more common in CM patients carrying an *MC1R* variant. Some earlier studies reported significant associations, which were not always confirmed, however, in subsequent investigations.[12–15] To summarize the available evidence on this topic, we performed a literature review and meta-analysis of the association between *MC1R* gene variants and the frequency of somatic mutations at the *BRAF*, *NRAS*, and *TERT* genes in CM patients.

2 | MATERIALS AND METHODS

We searched studies published until March 31st, 2020, in MEDLINE, EMBASE, Ovid Medline, OpenGrey (opengrey.eu), and GreyLit (greylit.org) using the following string: (melanocortin or mc1r) and (melanoma or "skin cancer") and (braf or b-raf or nras or ras or tert or telomerase or somatic or mutat*; study protocol accessible in the PROSPERO database,[16] registration number CRD42019136565). After a preliminary screening based on titles and abstracts, studies were read in a full copy to check their eligibility, and their reference lists were examined to find additional publications. No attempt was made to contact study authors for additional information. Studies were included if they reported (or allowed to calculate, e.g., from contingency tables) an odds ratio (OR) and corresponding 95% confidence intervals (CIs) for the association between *MC1R* status and the frequency of somatic mutations of the *BRAF*, *NRAS*, and/or *TERT* genes. In case of overlap, we considered the study with the largest size or (in case of equal size) the most adjusted one. When five or more independent OR for a given association were available, these were transformed into log OR and variance and pooled into a summary OR (SOR) using random effect models.[17,18] Between-estimates heterogeneity was assessed using the I^2 and the tau^2 statistics.[19,20] We used meta-regression to assess whether the SOR varied according to the study country and the year of publication; in addition, all study-specific OR stratified by tumor and patients' characteristics were reported in a dedicated table. Sensitivity analysis was conducted to examine the impact of removing each study at a time on the summary results. Finally, publication bias was assessed by applying the Macaskill test.[21] The study quality and risk of bias were assessed using the Q-Genie tool.[22,23] More methodological details are available as in the Supporting Information text.

Concerning *MC1R* status, different studies considered as "exposed" those subjects carrying any number, one, or two variants, of any, "R," or "r" type,[2] while wild-type subjects were always considered as "unexposed." In terms of somatic mutations, CM harboring mutations of the *BRAF* or *NRAS* genes were compared either to those wild-type at the same gene (the other not being taken into consideration), or to those that were *BRAF*/*NRAS* double wild-type (considering that mutations on those two genes are usually mutually exclusive in CM). These sources of diversity were accounted for by separately pooling ORs that were homogeneous in terms of both *MC1R* status and reference group for somatic mutations. Due to limited numbers, we disregarded ORs stratified by tumor or patients' characteristics and those focusing on different somatic mutations of a given gene.

Statistical analyses were conducted using SAS software version 9.4. All tests were two-sided and statistical significance was set at $\underline{p} < .05$.

3 | RESULTS

The literature search returned a total of 1852 nonduplicate entries: 1634 were excluded based on their title and abstract, and 218 full-text articles were assessed for eligibility, of which 12 met eligibility criteria and were retained[12–15,24–31]; no additional eligible paper was found by examining their reference lists (Figure S1). The main characteristics of the included studies are shown in Tables 1 and S1. Studies were conducted in Europe, Australia, and the United States, and encompassed a total of 3566 CMs mostly occurring on nonacral sites. The ORs that were entered in quantitative analyses were adjusted for age only or for age and gender, or were unadjusted (for five articles). The subset of Italian patients included in Landi et al.[12] was disregarded because of overlap with Fargnoli et al.[13] In Hacker et al.,[14] adjusted ORs were provided only separately for Spanish and Austrian patients, and these were entered as such in the analyses. Finally, all patients included in Pellegrini et al.[15] had multiple melanomas. All studies were of overall good quality (total score in the Q-Genie tool > 45), although some scored poorly in a few questions of the tool (Supporting Information text and Table S2).

There were five or more independent ORs for the association between *MC1R* status (any vs. no variant) and the presence of somatic mutations at *BRAF* and *NRAS* genes (for both, using as the group of reference either same-gene wild-type subjects, or *BRAF*/*NRAS* double wild-type subjects). Moreover, the frequency of *BRAF* somatic mutations (using same-gene wild-type individuals as reference) was compared between carriers of "R" (any number) versus no *MC1R* variants in five independent studies. The risk of *BRAF*-mutant CM was non-significantly increased by ≈20% among *MC1R* carriers (any or "R") when using *BRAF*-wt patients as a reference group, and decreased by a similar amount (again, without reaching

TABLE 1 Main characteristics of studies included in the literature review and meta-analysis on the association between germline *MC1R* variants and somatic *BRAF*, *NRAS*, and *TERT* mutations in melanomas

Author, year	Country	Years of diagnosis	No. patients	Skin site (%)[a]		Breslow thickness[b]	Somatic mutations	Adjusting variables
				Nonacral	Acral			
Landi, 2006[12]	United States[c]	2004–2005	112	100.0%	0.0%	Median 1.0 mm	*BRAF*	Age
Fargnoli, 2008[13]	Italy	1994–2002	177	100.0%	0.0%	Nr	*BRAF*	Age
Hacker, 2010[24]	Australia	1998–1999	123	100.0%	0.0%	74% < 0.75 mm	*BRAF*	None
Scherer, 2010[25]	Germany	Nr	202	93.6%	6.4%	Nr	*BRAF* and *NRAS*	Not specified
Thomas, 2010[26]	United States	Nr	213	99.1%	0.9%	56% < 0.75 mm	*BRAF*	Age and gender
Hacker, 2013[14]	Spain	2000–2009	134	100.0%	0.0%	23% < 0.75 mm	*BRAF* and *NRAS*	Age and gender
	Austria	1990–2008	241	100.0%	0.0%	54% < 0.75 mm	*BRAF* and *NRAS*	Age and gender
García-Casado, 2015[27]	Spain	2009–2012	230	100.0%	0.0%	Mean 2.2 mm	*BRAF* and *NRAS*	None
Hacker, 2016[28]	Australia	2007–2010	414	100.0%	0.0%	84% < 1.0 mm	*BRAF* and *NRAS*	Age and gender
Bruno, 2017[29]	Italy	Nr	93	100.0%	0.0%	Mean 1.6 mm	*BRAF*, *NRAS*, and *TERT*	None
Nagore, 20017[30]	Spain	2000–2014	356	100.0%	0.0%	48% < 1.5 mm	*TERT*	Age and gender
Thomas, 2017[31]	Australia and the United States	1998–2003	1227	100.0%	0.0%	Mean 0.7 mm	*BRAF* and *NRAS*	None
Pellegrini, 2018[15]	Italy	2010–2015	44[d]	100.0%	0.0%	Median 0.5 mm	*BRAF* and *TERT*	None

[a]No study included extra-cutaneous melanomas.

[b]Median or mean Breslow thickness reported when available; the percentage of melanoma below a given threshold otherwise.

[c]This study includes also 85 patients from Italy, which were, however, also included in the study by Fargnoli et al.,[13] and therefore not considered in the meta-analysis.

[d]This study includes 44 patients with multiple melanomas (35 and 9 patients with 2 and 3 melanomas, respectively).

statistical significance) while using double-wt patients as a reference group (Tables 2 and S3, Figures S2 and S3). No association was observed between *MC1R* variants and the risk of *NRAS*-mutant melanoma (Tables 2 and S3, Figures S4 and S5). The SOR never reached statistical significance (Table 2, Figures S2–S5). Between-studies heterogeneity was large ($I^2 > 50\%$) for SOR concerning the *BRAF* gene, but no significant associations were found in meta-regression analysis (Table S4), while the heterogeneity dropped to $I^2 = 0\%$ when excluding the two studies with most extreme estimates, that is, Fargnoli et al.[13] and Scherer et al.[25] There was no evidence of publication bias.

Associations for which there were four or fewer independent OR estimates are summarized in Table S5. *BRAF* mutations were significantly more frequent among *MC1R* variants carriers (any one, any two, and any two "r" vs. none) only in the study by Fargnoli et al.,[13] and less frequent (any "R" and any "r" vs. none *MC1R* variant) in the study by Scherer et al.[25] No study found significant associations between *MC1R* status and *NRAS* mutations. Only three studies

TABLE 2 Association between *MC1R* germline variants (any vs. none or any "R" vs. none) and the frequency of somatic mutations of the *BRAF* and *NRAS* genes (group of reference: same-gene wild-type or *BRAF*/*NRAS* double wild-type) in melanomas

MC1R germline variants	Somatic mutations	*N*	OR[a]	95% CI[b]	I^2	Tau^2
Any versus none	*BRAF* mutated versus *BRAF* wt	11	1.20	0.70–2.07	68%	0.44 (*SE* 0.29)
Any "R" versus none	*BRAF* mutated versus *BRAF* wt	5	1.18	0.28–4.95	96%	0.15 (*SE* 0.16)
Any versus none	*BRAF* mutated versus *BRAF*/*NRAS* wt/wt	6	0.78	0.38–1.59	57%	0.30 (*SE* 0.32)
Any versus none	*NRAS* mutated versus *NRAS* wt	7	0.98	0.62–1.56	0%	0.00 (*SE* 0.12)
Any versus none	*NRAS* mutated versus *BRAF*/*NRAS* wt/wt	6	0.93	0.52–1.65	0%	0.00 (*SE* 0.16)

[a]OR, odds ratio.

[b]CI, confidence interval.

focused on *TERT* mutations, all of which reported positive associations with the presence of *MC1R* germline variants, consistently for different types and numbers of variants, with ORs often above 2.00 and which achieved statistical significance in Nagore et al.[30] More results are available in the Supporting Information material.

4 | DISCUSSION

This review and meta-analysis aimed to better investigate the interplay between germline and somatic mutations in key genetic drivers of CM. Our pooled risk estimates, based on over 3500 CM cases from 12 independent studies conducted between 2006 and 2018, showed moderate, yet the nonstatistically significant association between *MC1R* variants and *BRAF/NRAS* mutant melanomas. On the contrary, we highlighted a consistently positive association between *MC1R* variants and *TERT* promoter mutations, although based on only three studies.

Study-specific ORs linking *MC1R* variants and *BRAF* mutations varied considerably across studies, with two even reporting either direct[13] or inverse[25] significant associations. This heterogeneity might partly reflect differences across studies in terms of inclusion criteria, classification method of *MC1R* gene variants, or samples used for DNA extraction and mutational analysis (e.g., paraffin-embedded tissue vs. fresh or frozen tissue). Despite early claims of significant associations, our findings suggest that the frequency of *BRAF* and *NRAS* mutations in melanoma is unlikely to substantially differ according to the patient's *MC1R* status, although we cannot rule out that moderate associations do exist, which failed, however, to achieve statistical significance because of the still limited sample size. A similar argument may apply to associations limited to specific CM subtypes (e.g., those on skin not showing chronic sun-induced damage, as in Landi et al.[12]).

The number of studies investigating the relationship between *MC1R* and *TERT* was limited, yet in our opinion those results deserve attention. Germline *MC1R* variants affect the mutational landscape of CM, especially increasing the frequency of UV-induced C > T mutations (although non-C > T mutations are also increased, possibly via oxidative DNA damage).[32,33] *TERT* promoter mutations are common in CM development (19%–41% in the three studies reviewed here, in line with literature data) and typically carry the UV-signature, thus not surprisingly they seem more common among *MC1R* variant carriers, who have vary degrees of impairment in pigmentary function and reduced protection against UV light-induced damage.

In conclusion, we did not find evidence of a major association between *MC1R* status and *BRAF/NRAS* mutations in CM, but suggested that the occurrence of *TERT* promoter mutations could be favored by the presence of *MC1R* germline variants. The latter finding warrants confirmation given the few studies published to date, yet it provides intriguing clues for further research aimed at elucidating the relationship between genetic predisposition and mutations of specific genes in CM development.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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