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Letter to the Editor

MC1R variation and melanoma risk in relation to host/clinical and environmental factors in *CDKN2A* positive and negative melanoma patients

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Abstract: Host, environmental and genetic factors differently modulate cutaneous melanoma (CM) risk across populations. Currently, the main genetic risk determinants are germline mutations in the major known high-risk susceptibility genes, *CDKN2A* and *CDK4*, and variants of the low-risk gene *MC1R*, which is key in the pigmentation process. This case–control study aimed at investigating the influence of the main host and environmental risk factors and of *MC1R* variation on CM risk in 390 *CDKN2A*-negative and 49 *CDKN2A*-positive Italian individuals. Multivariate analysis showed that *MC1R* variation, number of nevi and childhood sunburns doubled CM risk in *CDKN2A*-negative individuals. In *CDKN2A*-positive individuals, family history of CM and presence of atypical nevi, rather than *MC1R* status, modified risk (20.75- and 2.83-fold, respectively). Occupational sun exposure increased CM risk (three to sixfold) in both *CDKN2A*-negative and *CDKN2A*-positive individuals, reflecting the occupational habits of the Ligurian population and the geographical position of Liguria.

Key words: CDKN2A – cutaneous melanoma – MC1R – nevi – risk factors

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Background

An increased risk of cutaneous melanoma (CM) is associated with the presence of numerous melanocytic and/or atypical nevi. Exposure to solar UV radiation is a key environmental risk factor (1–3), while the main genetic risk determinants are germline mutations in the major known susceptibility genes, *CDKN2A* and *CDK4*, and in variants of the low-risk gene *MC1R*, which plays a key role in the pigmentation process (4–6).

Penetrance of *CDKN2A* mutations in CM families is incomplete and variable (7). Such variations may be associated with individual and environmental risk factors, and with the impact of low-risk genes such as *MC1R*.

MC1R variants are associated with red hair, pale skin, freckling and sun sensitivity and have been found to increase CM risk regardless of familiality (8–15). Furthermore, it has been suggested that *MC1R* variants may be involved in CM development both via pigmentary and non-pigmentary pathways (8) and in increased CM risk in families with *CDKN2A* mutations (9,16–20).

An international study recently found that *MC1R* variants, hair colour and number of nevi were jointly associated with CM risk in *CDKN2A* mutation carriers. Separate observation of data from the Italian families we contributed did not show the association (21), confirming the results of a previous study on carriers of the common *CDKN2A* G101W founder mutation, which showed an impact of *MC1R* variants on CM risk in G101W carriers from Spain, France and the United States but not in G101W carriers from Italy (17).

Questions addressed

The distribution and relative weight of the genetic factors involved in CM susceptibility appear to vary across populations, and few epidemiological case–control studies have been conducted in Italy to clarify their role (12,22–25). We have therefore investigated the influence of the main host and environmental risk factors as well as of *MC1R* variation on CM risk in *CDKN2A*-negative (*CDKN2A*−) and *CDKN2A*-positive (*CDKN2A*+) Italian patients with CM.

Experimental design

This is a case–control study: 439 histologically confirmed CM cases and 490 controls were interviewed on their family history, host and environmental risk factors and genotyped for CDKN2A and MC1R. The risk of developing CM associated with the phenotypic characteristics, sun exposure and FH of nevi and of CM and with the presence of MC1R variants was estimated by means of the odds ratio (OR) (Data S1). The study was approved by the Ethics Committees of the participating Institutions, and written informed consent was obtained from all cases and controls.

Results

Novel CDKN2A and CDK4 mutations

Forty-nine of the 439 CM cases (11.2%) were *CDKN2A* mutation carriers. The mutations found were 39 p.G101W, 6 p.E27X, 1 p. P48T, 1 p.R58X, 1 p. A127P (all of which we have previously described except 1 p. E27X; 26,27) and two novel mutations, which we found in two non-familial patients. One is a novel *CDKN2A* germline mutation (+2 T>G starting from the first ATG;

Table 1. Effect of *MC1R* variants on melanoma risk (univariate analysis)

	Controls	Cases CDKN2A-negative	Crude OR	Cases CDKN2A-positive	Crude OR
wt/wt	185 (37.8)	102 (26.2)	1 ref	14 (28.6)	1 ref
1 MC1R variant	236 (48.2)	192 (49.2)	1.48 (1.08–2.01)*	24 (49.0)	1.34 (0.68–2.67)
>2MC1R variants	69 (14.1)	96 (24.6)	2.52 (1.70–3.74)**	11 (22.4)	2.11 (0.91–4.86)
r/r r/wt	212 (43.3)	135 (34.6)	1.15 (0.83–1.60)	17 (34.7)	1.06 (0.51–2.21)
R/r R/wt R/R	93 (19.0)	153 (39.2)	2.98 (2.10–4.25)**	18 (36.7)	2.56 (1.22–5.37)*

The *P* values are indicated as follows: $*P < 0.05$; $**P < 0.0001$.

p.M1R) that affects the beginning of p16, the other is the rare R24C in *CDK4* that was identified in a non-familial, single patients with CM (28).

Compared to *CDKN2A*− cases, *CDKN2A*+ cases were younger and displayed a higher frequency of multiple CMs (Table S1).

Phenotype, risk factors and CM

FH of CM increased risk of CM in both *CDKN2A*− (OR = 5.31; 95% CI 1.97–14.31; $P = 0.001$) and *CDKN2A*+ individuals (OR = 71.22; 95% CI 22.95–220.98; $P < 0.0001$), while FH of cutaneous nevi increased the risk of CM among *CDKN2A*+ cases only (OR = 2.44; 95% CI 1.25–4.54; $P = 0.008$). Established phenotypic risk factors, with the exception of eye colour, as well as a history of blistering sunburns at a very young age (<10 years) and continuous occupational sun exposure, were associated with an increased risk of CM in both *CDKN2A*− and *CDKN2A*+ cases; regular sunscreen use decreased the risk (Table S2). The presence of ≥ 10 nevi was associated with a twofold increased risk in both *CDKN2A*− and *CDKN2A*+ cases. Clinically, atypical nevi were associated with a fourfold increased risk in *CDKN2A*+ cases (Table S2).

MC1R variants and CM

Table 1 shows the frequency of MC1R variants in cases and controls: the presence of ≥ 2 variants or ≥ 1R variant increased the risk of CM in both *CDKN2A*− and *CDKN2A*+ cases. However, the presence of *MC1R* variants did not significantly modify the risk of CM across the categories of the examined phenotypic characteristics and sun exposure according to *CDKN2A* status (Tables S3 and S4). Individuals who reported sun exposure during vacations and outdoor activities at any age and carried ≥ 2 *MC1R* and R variants had a three to fivefold increased risk of CM compared to non-carriers.

A multivariate logistic analysis confirmed the role of *MC1R* as independent risk factor for *CDKN2A*− cases only. Skin phototype, occupational sun exposure and sunburns at a very young age were confirmed as independent risk factors for both *CDKN2A*− and *CDKN2A*+ cases (Table 2).

Conclusions

The results of this study confirm previous findings in our population, showing that Italian *CDKN2A*+ CM cases have substantially fewer *MC1R* variants than cases from other geographic areas and that Italian cases do not show an association between *MC1R* and CM risk (17,21). Our findings suggest that other genes, besides *MC1R*, may modify CM risk in *CDKN2A* + cases and that *MC1R* plays a role in CM development in *CDKN2A*− cases both via pigmentary and non-pigmentary pathways. In *CDKN2A*+ individuals, a positive FH of CM and presence of atypical nevi, rather than *MC1R* status, modified disease risk. The number of acquired melanocytic nevi increased the risk of CM in *CDKN2A*− individuals. Interestingly, occupational sun exposure increased CM risk in both *CDKN2A*− and *CDKN2A*+ individuals, reflecting the occupational habits of the Ligurian population and the geographical position of our region, which is a narrow strip of cultivated land lying on the Mediterranean Sea. This finding is especially of value when analyzed jointly with *MC1R* variation (29), which has been described to be preferentially associated with CM in non-chronic sun-damaged areas, in concert with *BRAF* mutation.

Overall, as different host/clinical, environmental and genetic characteristics emerged as independent risk factors in *CDKN2A*+ and *CDKN2A*− CM cases, our results have potential implications for genetic counselling.

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Table 2. Risk factors associated with cutaneous melanoma (CM) susceptibility in CDKN2A− and CDKN2A+ cases

Risk factors	*CDKN2A*− Adj OR (95% CI)	*CDKN2A*+
1st degree FH of CM		
No	1 ref	1 ref
Yes	Removed	20.75 (5.52–77.98)
Total nevus count		
<10	1 ref	1 ref
≥10	1.97 (1.31–2.94)**	Removed
Clinically atypical nevi		
No	1 ref	1 ref
Yes	Removed	2.83 (1.01–7.90)*
Skin phototype		
I–II	1 ref	1 ref
III	1.45 (0.92–2.29)	1.98 (0.70–5.58)
IV	0.31 (0.17–0.55)***	0.35 (0.08–1.50)
Sun exposure ≥ 15 years		
Occupational		
Seldom	1 ref	1 ref
Often	3.14 (1.29–7.65)*	6.06 (1.31–27.95)*
Always	5.84 (2.08–16.45)**	6.86 (1.20–39.26)*
Sunburns < 10 years		
No	1 ref	1 ref
Yes	2.40 (1.35–4.29)**	3.28 (0.95–11.31)
Use of sunscreens		
Seldom	1 ref	1 ref
Often	0.57 (0.34–0.98)*	Removed
Always	0.37 (0.22–0.63)***	Removed
MC1R variant		
wt	1 ref	1 ref
r	1.02 (0.64–1.62)	Removed
R[1]	2.08 (1.22–3.54)**	Removed

The *P* values are indicated as follows: $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$.
[1]*MC1R* variants associated with functional impairment of MC1R: D84E, R151C, R160W, D294H, Y152X, R142H and I155T.

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Authorship

PG and LBo designed the research study, analyzed and interpreted data and wrote the manuscript. PG, LP, SN, VA performed the research. PG and LP managed data. WB MB MG PQ recruited patients and controls and acquired data. LBa contributed to article preparation and editing. GBS contributed essential reagents and together with PQ conceived and supervised the study.

Conflict of interest

The authors declare no conflict of interest.

References

1 Gandini S, Sera F, Cattaruzza M S *et al.* Eur J Cancer 2005: **41**: 28–44. Review.
2 Gandini S, Sera F, Cattaruzza M S *et al.* Eur J Cancer 2005: **41**: 45–60. Review.
3 Gandini S, Sera F, Cattaruzza M S *et al.* Eur J Cancer 2005: **41**: 2040–2059. Review.
4 Lin J, Hocker T L, Singh M *et al.* Br J Dermatol 2008: **159**: 286–291.
5 Pho L, Grossman D, Leachman S A. Curr Opin Oncol 2006: **18**: 173–179. Review.
6 Raimondi S, Sera F, Gandini S *et al.* Int J Cancer 2008: **122**: 2753–2760.
7 Bishop J A, Wachsmuth R C, Harland M *et al.* J Invest Dermatol 2000: **114**: 28–33.
8 Bastiaens M T, ter Huurne J A, Kielich C *et al.* Am J Hum Genet 2001: **68**: 884–894.
9 Box N F, Duffy D L, Chen W *et al.* Am J Hum Genet 2001: **69**: 765–773.
10 Höiom V, Tuominen R, Käller M *et al.* Pigment Cell Melanoma Res 2009: **22**: 196–204.
11 Kanetsky P A, Panossian S, Elder D E *et al.* Cancer 2010: **116**: 2416–2428.
12 Landi M T, Kanetsky P A, Tsang S *et al.* J Natl Cancer Inst 2005: **97**: 998–1007.
13 Ibarrola-Villava M, Fernandez L P, Pita G *et al.* Exp Dermatol 2010: **19**: 836–844.
14 Palmer J S, Duffy D L, Box N F *et al.* Am J Hum Genet 2000: **66**: 176–186.
15 Stratigos A J, Dimisianos G, Nikolaou V *et al.* J Invest Dermatol 2006: **126**: 1842–1849.
16 Chaudru V, Laud K, Avril M F *et al.* Cancer Epidemiol Biomarkers Prev 2005: **14**: 2384–2390.
17 Goldstein A M, Chaudru V, Ghiorzo P *et al.* Int J Cancer 2007: **121**: 825–831.
18 Goldstein A M, Landi M T, Tsang S *et al.* Cancer Epidemiol Biomarkers Prev 2005: **14**: 2208–2212.
19 van der Velden P A, Sandkuijl L A *et al.* Am J Hum Genet 2001: **69**: 774–779.
20 Fargnoli M C, Gandini S, Peris K *et al.* Eur J Cancer 2010: **46**: 1413–1420. Review.
21 Demenais F, Mohamdi H, Chaudru V *et al.* J Natl Cancer Inst 2010: **102**: 1568–1583.
22 Fargnoli M C, Altobelli E, Keller G *et al.* Melanoma Res 2006: **16**: 175–182.
23 Landi M T, Baccarelli A, Calista D *et al.* Br J Cancer 2001: **85**: 1304–1310.
24 Naldi L, Altieri A, Imberti G L *et al.* Cancer Causes Control 2005: **16**: 893–899.
25 Chiarugi A, Nardini P, Crocetti E *et al.* J Eur Acad Dermatol Venereol 2012: **26**: 194–199.
26 Ghiorzo P, Gargiulo S, Pastorino L *et al.* Hum Mol Genet 2006: **15**: 2682–2689.
27 Pastorino L, Bonelli L, Ghiorzo P *et al.* Pigment Cell Melanoma Res 2008: **21**: 700–709.
28 Zuo L, Weger J, Yang Q *et al.* Nat Genet 1996: **12**: 97–99.
29 Landi M T, Bauer J, Pfeiffer R M *et al.* Science 2006: **313**: 521–522.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Table S1. Clinical characteristics of CM cases according to *CDKN2A* status (− or +).

Table S2. Risk of CM associated with family history of nevi and of melanoma, phenotypic characteristics and sun exposure according to *CDKN2A* status (age and sex-adjusted analyses).

Table S3. Effect of *MC1R* variants on CM risk stratified by phenotypic characteristics (univariate analysis).

Table S4. Effect of *MC1R* variants on CM risk according to sun exposure.

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