**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

Accepted for publication 4 June 2012

**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 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 pigmentedary and non-pigmentedary 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;
and displayed a higher frequency of multiple CMs (Table S1).

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 an independent risk factor for CDKN2A− cases only. Skin phenotype, 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 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.

**Acknowledgements**

We are indebted to the patients and families whose generous participation made this study possible. We are grateful to Sara Gliori, MD, for examining 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.

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

<table>
<thead>
<tr>
<th>MC1R–MC2R</th>
<th>Controls</th>
<th>Cases CDKN2A-negative</th>
<th>Cases CDKN2A-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>185 (37.8)</td>
<td>102 (26.2)</td>
<td>1 ref</td>
</tr>
<tr>
<td>1 MC1R variant</td>
<td>236 (48.2)</td>
<td>192 (49.2)</td>
<td>1.48 (1.08–2.01)</td>
</tr>
<tr>
<td>&gt;2MC1R variants</td>
<td>69 (14.1)</td>
<td>96 (24.6)</td>
<td>2.52 (1.70–3.74)**</td>
</tr>
<tr>
<td>R/R</td>
<td>212 (43.3)</td>
<td>135 (34.6)</td>
<td>1.15 (0.83–1.60)</td>
</tr>
<tr>
<td>R/R R/Vwt R/R</td>
<td>93 (19.0)</td>
<td>153 (39.2)</td>
<td>2.98 (2.10–4.25)**</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>CDKN2A−</th>
<th>CDKN2A+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>1 ref</td>
<td>1 ref</td>
</tr>
<tr>
<td>Yes</td>
<td>Removed</td>
<td>20.75 (5.52–77.98)</td>
</tr>
<tr>
<td>Total nevus count</td>
<td>1 ref</td>
<td>1 ref</td>
</tr>
<tr>
<td>&lt;10</td>
<td>1 ref</td>
<td>1 ref</td>
</tr>
<tr>
<td>≥10</td>
<td>1.97 (1.31–2.94)**</td>
<td>Removed</td>
</tr>
<tr>
<td>Clinically atypical nevi</td>
<td>No</td>
<td>1 ref</td>
</tr>
<tr>
<td>Yes</td>
<td>Removed</td>
<td>2.83 (1.01–7.90)**</td>
</tr>
<tr>
<td>Skin phenotype</td>
<td>II</td>
<td>1 ref</td>
</tr>
<tr>
<td>III</td>
<td>1.45 (0.92–2.29)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0.31 (0.17–0.55)**</td>
<td></td>
</tr>
<tr>
<td>Sun exposure ≥15 years</td>
<td>Occupational Seldom</td>
<td>1 ref</td>
</tr>
<tr>
<td>Often</td>
<td>3.14 (1.29–7.65)*</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>5.84 (2.08–16.45)**</td>
<td></td>
</tr>
<tr>
<td>Sunburns &lt;10 years</td>
<td>No</td>
<td>1 ref</td>
</tr>
<tr>
<td>Yes</td>
<td>2.40 (1.35–4.29)**</td>
<td></td>
</tr>
<tr>
<td>Use of sunscreens</td>
<td>Seldom</td>
<td>1 ref</td>
</tr>
<tr>
<td>Often</td>
<td>0.57 (0.34–0.98)*</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>0.37 (0.22–0.63)**</td>
<td></td>
</tr>
<tr>
<td>MC1R variant</td>
<td>wt</td>
<td>1 ref</td>
</tr>
<tr>
<td>R</td>
<td>1.02 (0.64–1.62)</td>
<td></td>
</tr>
<tr>
<td>R/R</td>
<td>2.08 (1.22–3.54)**</td>
<td></td>
</tr>
</tbody>
</table>

The P values are indicated as follows: *P < 0.05; **P < 0.001; ***P < 0.0001.

1MC1R variants associated with functional impairment of MC1R: D84E, R151C, R160W, D294H, Y152X, R142H and I155T.
ing patients and controls and to Associazione Mater Matuta for their help in recruiting healthy volunteers. This study was funded by Fondazione CARIGE 2010, PRIN 2008 to G.B.-S, ACM and IMI fellowship funded Erme Rouge onlus in memory of Mara Naum. The study was approved by the Genoa IST (National Cancer Institute) Ethical Committee on July 25, 2001, code OM01009 and by the Genoa San Martino University Hospital Ethical Committee on May 28, 2001, code 5k/2001.

**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 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**


**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 (−/+).
- **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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