**INTRODUCTION**

Basal cell carcinoma (BCC) is the commonest cancer in the Caucasian population, accounting for 29% of all cancers (DePinho, 2000). Its incidence is increasing (Wong et al., 2003), and it constitutes an important public health problem.

Solar UVR has been shown to be the main environmental causative factor of BCC (Kricker et al., 1994). Other BCC risk factors include phenotypic traits such as red hair, freckles, fair skin that does not readily tan, and a family history of skin cancer (Naldi et al., 2000).

Genetic factors also mediate BCC risk.

Basal cell nevus syndrome, an autosomal dominant disorder comprising developmental defects and multiple BCCs, has been linked to inactivating mutations in the patched (PTCH) gene, shedding some light on the crucial importance of the PTCH/sonic hedgehog pathway in BCC pathogenesis (Hahn et al., 1996; Gorlin, 2004). In addition, recent data has suggested that PTCH polymorphism may also be associated with BCC risk (Strange et al., 2004a, b; Asplund et al., 2005). Notably, the PTCH variant c. 3944C (1315Pro) may confer an increased population risk for BCC and an increased individual risk for multiple BCC (Asplund et al., 2005).

BCC susceptibility has also been shown to be mediated by defects in the nucleotide excision repair system (either severe deleterious mutations giving rise to xeroderma pigmentosum (Magnaldo and Sarasin, 2004), or less deleterious variants in XPD or ERCC2 genes (Han et al., 2004; Lovatt et al., 2005)). In addition, BCC formation is also influenced by polymorphism of genes encoding detoxifying enzymes (glutathione S-transferase, cytochrome P450) (Lear et al., 1996; Ramachandran et al., 2001), and in a key gene involved in pigmentation, the melanocortin 1 receptor (MC1R) (Bastiaens et al., 2001; Box et al., 2001).

**MC1R** and **PTCH** Gene Polymorphism in French Patients with Basal Cell Carcinomas

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In this study, we assessed the role of melanocortin 1 receptor (MC1R) variants and of two patched (PTCH) polymorphisms (c.3944C>T (P1315L), insertion 18 bp IVS1-83) as risk factors for basal cell carcinoma (BCC) in the French population. The population investigated comprised 126 BCC patients who were enrolled on the basis of specific criteria (multiple and/or familial BCC and/or onset before the age of 40 years and/or association with another tumor) – and 151 controls matched for ethnicity, age, and sex. MC1R variants appeared as a moderate risk factor for BCC (odds ratio (OR) for one and two variants, 2.17 [1.28–3.68] and 7.72 [3.42–17.38], respectively), independently of pigmentation characteristics (OR = 2.53 [1.34–4.48]). Interestingly, in addition to the predictable red hair color (RHC) alleles, two non-RHC alleles (V60L and V92M) were also closely associated with BCC risk (OR 3.21 [1.91–5.38] and 2.87 [1.5–5.48], respectively), which differs from the situation in the Celtic population. In addition, the PTCH c.3944C/C genotype was also associated with BCC risk (OR 1.94 [1.2–3.1]), especially in the subgroup of patients with multiple tumors (OR 2.16 [1.3–3.6]). Thus, our data show that MC1R and PTCH variants are associated with BCC risk in the French population. We further suggest that assessing MC1R and PTCH status could be useful, combined with the assessment of clinical risk factors, in identifying high-risk patients to be targeted for prevention or more rigorous surveillance.

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Abbreviations: -MSH, a-melanocyte-stimulating hormone; BCC, basal cell carcinoma; CI, confidence interval; MC1R, melanocortin 1 receptor; OR, odds ratio; PTCH, patched; RHC, red hair color

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pigment eumelanin rather than of the non-protective red pigment pheomelanin (Sturm et al., 2003b). MC1R is highly polymorphic in Caucasian populations, and numerous MC1R variants have been demonstrated to lead to a loss of function, decreasing either cAMP production or α-MSH binding affinity (Frandberg et al., 1998; Schioth et al., 1999; Healy et al., 2001; Jimenez-Cervantes et al., 2001a, b; Ringholm et al., 2004).

Four MC1R variants alleles (R142H, R151C, R160W, and D294H) have been shown to be associated with the red hair and fair skin phenotype (red hair color (RHC)) that is characterized by fair pigmentation (fair skin, red hair and freckles) and sun sensitivity (Sturm, 2002; Rees, 2004). Subsequently, loss-of-function variants of MC1R have been shown to play an important role in determining the risks of melanoma and non-melanoma-skin cancer (reviewed in Sturm et al., 2003a). Three studies in particular have previously demonstrated that there is an association between BCC risk in populations of Celtic origin with three MC1R RHC alleles (R151C, R160W, D294H) (Bastiaens et al., 2001; Box et al., 2001; Dwyer et al., 2004).

The aim of this work was to assess simultaneously the role of MC1R and of PTCH polymorphisms as BCC risk factors in an ethnically-distinct population by means of a case control-study.

RESULTS

Clinical BCC risk factors

The clinical characteristics of all BCC patients and controls are summarized in Table 1. All the classical clinical risk factors (skin types I–II, light eye and hair colors, and solar lentigines) were strongly associated with BCC (P≤0.0001, odds ratio (OR) ranging from 2.98 to 4.91).

Frequency of MC1R variants and effect on BCC risk

Nineteen non-synonymous MC1R variant alleles were identified (Table 2), 15 of which were classified as functional (see Materials and Methods and Table 2). These included three previously unreported MC1R variants (N118K, N281S, and R306H).

Initially, in order to study the effect of MC1R, all “functional” variants (RHC and non-RHC) were pooled (Table 2). MC1R variants were significantly more frequent in the BCC patients, and there was a gene dosage effect on BCC risk (OR of 2.17 for one variant and 7.72 for two variants, respectively, Table 3a).

When detailing our results, we observed that three variants (two non-RHC: V60L and V92M and one RHC: R151C) were significantly associated with BCC risk. One other RHC allele (D294H) showed a nearly significant association, whereas the two other RHC alleles (R142H, R160W) were not associated with BCC risk (Table 2).

When RHC and non-RHC alleles were assessed separately, we observed that both non-RHC and RHC alleles were significantly associated with BCC risk (Table 3b).

Persistence of MC1R effect after stratification for pigmentation characteristics

Table 4 shows the significant persistence of BCC risk according to MC1R variants after stratification for all categories of pigmentation characteristic (skin type, hair color, eye color, solar lentigines). Notably, when two MC1R variants were present, the risk of BCC was present both in dark- and fair-pigmented individuals. When only one MC1R variant was present, the effect was less pronounced. These findings strongly suggest that MC1R variants constitute independent risk factors for the development of BCC.

Distribution of MC1R variants in the different BCC subgroups

The prevalence of MC1R variants ranged from 63 to 80% among the BCC subgroups (multiple BCC, BCC before the age of 40 years, familial BCC, BCC associated with another cancer), but there was no statistical difference between these different categories. In addition, MC1R variant frequency was not statistically different in patients with BCC localized only

| Table 1. Clinical characteristics of patients with basal cell carcinomas and controls |
|-----------------------------------|----------|----------|-------|
| Clinical characteristics          | BCC (126) | Controls (151) | P   | OR   |
| Gender                           |          |            |   |     |
| Women                            | 71 (56)  | 84 (56)    | 0.86| NA |
| Men                              | 55 (44)  | 67 (44)    |     |     |
| Mean age (years)                 | 48 [24–91]| 50 [19–100]  | NA |
| Skin type                        |          |            |   |     |
| III                              | 33 (33)  | 62 (41)    | Ref.1 | 2.98 [1.77–5.01] |
| IV                               | 3 (3)    | 33 (22)    |     |     |
| I                                | 11 (11)  | 6 (4)      | <0.0001|     |
| II                               | 54 (53)  | 50 (33)    |     |     |
| Hair color                       |          |            |   |     |
| Dark                             | 17 (19)  | 47 (31)    | Ref.1 |     |
| Dark brown                       | 23 (24)  | 60 (40)    |     |     |
| Blond                            | 21 (23)  | 10 (7)     | 0.0001| 3.38 [1.97–5.82] |
| Light brown                      | 28 (30)  | 29 (19)    |     |     |
| Red                              | 4 (4)    | 4 (3)      |     |     |
| Eye color                        |          |            |   |     |
| Dark                             | 36 (37)  | 97 (65)    | Ref. |     |
| Light                            | 61 (63)  | 53 (35)    | <0.0001| 3.1 [1.83–5.26] |
| Lentigines                       |          |            |   |     |
| No                               | 28 (29)  | 97 (67)    | Ref. |     |
| Yes                              | 68 (71)  | 48 (33)    | <0.0001| 4.91 [2.81–8.57] |

OR=odds ratios are indicated with 95% CI; NA=not applicable; Ref.=reference.

1OR calculation was performed by comparing light versus dark hair color, and skin type I-II versus III-IV. Significant associations are shown in bold characters.
on sun-exposed sites (head and neck, limbs) or only on a less-exposed site (trunk) (data not shown).

Distribution of \textit{PTCH} polymorphisms among cases and controls
The distribution of genotypes of the \textit{PTCH} single nucleotide polymorphism rs357564 is shown in Table 5. This single nucleotide polymorphism was in Hardy–Weinberg equilibrium in both patients and controls, and showed a significantly different distribution in BCC patients and controls. Overall, the c.3944C/C genotype was significantly more frequent in patients than in controls (51 vs 35\%, \textit{P}-value = 0.007, OR 1.94 [1.2–3.1]).

Furthermore, this genotype was also more frequent in the subgroup of patients with multiple BCCs (\textit{P} = 0.003, OR 2.16 [1.3–3.6]), but not in that with a single BCC (\textit{P} = 0.70). The association with multiple tumors persisted in the subgroup of 15 patients with more than five tumors (Fisher’s exact test = 0.024). On the other hand, this association was not

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### Table 2. Allelic frequencies of \textit{MC1R} variants in BCC patients and control subjects

<table>
<thead>
<tr>
<th>\textit{MC1R} consensus</th>
<th>\textbf{Controls (n=302)}</th>
<th>\textbf{BCC (n=252)}</th>
<th>\textbf{P}</th>
<th>\textbf{OR [95% CI]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC1R consensus type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R163Q</td>
<td>10 (4)</td>
<td>8 (2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T95M</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V180L</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R223Q</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MC1R wt</td>
<td>135 (53.6)</td>
<td>230 (76.5)</td>
<td></td>
<td>Ref. 1</td>
</tr>
<tr>
<td>MC1R variants type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V60L\textsuperscript{1,2}</td>
<td>49 (19.4)</td>
<td>26 (8.6)</td>
<td>\textbf{0.0001}</td>
<td>\textbf{3.21 [1.91–5.38]}</td>
</tr>
<tr>
<td>S83P\textsuperscript{2,3}</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>D84E\textsuperscript{2,4}</td>
<td>0</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>V92M\textsuperscript{5}</td>
<td>27 (10.7)</td>
<td>16 (5.3)</td>
<td>\textbf{0.001}</td>
<td>\textbf{2.87 [1.5–5.48]}</td>
</tr>
<tr>
<td>V92L\textsuperscript{5}</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>N118K\textsuperscript{5}</td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>V122M\textsuperscript{5}</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>R142H\textsuperscript{1,3,5}</td>
<td>4 (1.6)</td>
<td>2 (0.7)</td>
<td>0.09\textsuperscript{6}</td>
<td>3.40 [0.62–18.8]</td>
</tr>
<tr>
<td>R151C\textsuperscript{1,4}</td>
<td>14 (5.5)</td>
<td>5 (1.6)</td>
<td>\textbf{0.0007}\textsuperscript{6}</td>
<td>\textbf{5.11 [1.82–14.4]}</td>
</tr>
<tr>
<td>I155T\textsuperscript{2,3}</td>
<td>2 (0.8)</td>
<td>2 (0.7)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>R160W\textsuperscript{1,2,4}</td>
<td>11 (4.4)</td>
<td>11 (3.6)</td>
<td>0.22</td>
<td>1.66 [0.7–3.93]</td>
</tr>
<tr>
<td>N281S\textsuperscript{3}</td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>D294H\textsuperscript{2,3}</td>
<td>7 (2.7)</td>
<td>4 (1.3)</td>
<td>0.053\textsuperscript{6}</td>
<td>2.98 [0.86–10.37]</td>
</tr>
<tr>
<td>R306H\textsuperscript{3}</td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ins 86\textsuperscript{7}</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total variants type II</td>
<td>117 (46.4)</td>
<td>72 (23.5)</td>
<td>\textbf{&lt;0.0001}</td>
<td>\textbf{2.77 [1.93–3.97]}</td>
</tr>
</tbody>
</table>

\textsuperscript{1} MC1R variants are unable to stimulate cAMP production as strongly as the wild-type receptor in response to \textalpha-MSH stimulation (Frandberg \textit{et al}., 1998; Healy \textit{et al}., 2001; Jimenez-Cervantes \textit{et al}., 2001a, b; Schioth \textit{et al}., 1999).
\textsuperscript{2} MC1R variants previously shown to be associated with the RHC phenotype (reviewed in (Sturm, 2002)).
\textsuperscript{3} MC1R variants predicted to be damaging by the Polyphen program.
\textsuperscript{4} MC1R variants previously shown to be associated with melanoma risk (Kennedy \textit{et al}., 2001; Palmer \textit{et al}., 2000; Valverde \textit{et al}., 1996).
\textsuperscript{5} MC1R variants showing a decreased \textalpha-MSH-binding affinity (Jimenez-Cervantes \textit{et al}., 2001a).
\textsuperscript{6} Fisher’s exact test.
\textsuperscript{7} MC1R single nucleotide insertion producing a frameshift mutation that results in a prematurely terminated, non-functioning receptor.
seen either in patients who developed BCC before the age of 40 years or in patients whose BCC was associated with another tumor.

We also observed that the c.3944C/C genotype was slightly less frequent in controls with light hair color  

\( P = 0.04 \), OR 0.44 [0.2–0.98]), but was not associated with skin type or eye color. Importantly, the BCC risk attributable to the c.3944C/C genotype persisted after stratification for fair/light color  

\( P = 0.006 \), OR 3.4 [1.42–8.24]) and skin types I–II  

\( P = 0.028 \), OR 2.27[1.1–4.73]).
The distribution of genotypic frequencies of the second PTCH polymorphism, ins18 bp 4IVS1-84, was not statistically different in the cases and controls ($P=0.42$).

**Interaction between MC1R and PTCH variants on BCC risk**

To test the interaction between MC1R and PTCH variants on BCC risk, we considered as BCC risk factors the c.3944C/C genotype of PTCH and functional MC1R variants as defined in Materials and Methods. We then tested whether MC1R genotype depends on PTCH genotype in the BCC patients. The c.3944C/C genotype frequency was not significantly different between BCC patients carrying a MC1R variant and those that did not carry one (54% vs 46% $P=0.45$), suggesting an independent effect of both genes on BCC risk. However, the limited sizes of each subgroup need to take these results cautiously.

**Multiple logistic regression analyses**

Interestingly, in multiple logistic regression analyses, which take into account all these potential confounders, the presence of MC1R variants remained an important BCC risk factor (Table 6), together with fair pigmentation characteristics and poor tanning response to UVR.

**DISCUSSION**

In this study, we have analyzed for the first time the distribution of both MC1R variants and of two PTCH polymorphisms in the French population. Our results confirm the association of MC1R variants with the risk of developing BCC in this population. Yet, a certain number of differences from previous studies should be pointed out. In our population, both non-RHC and RHC alleles were closely associated with BCC risk (Table 3). The V60L and V92M variants that have been reported to be low-penetrant RHC alleles (Sturm et al., 2003b) and that are only slightly or not at all associated with non-melanoma-skin cancer risk in the Celtic population (Jones et al., 1999; Box et al., 2001) were the most frequently observed variants in our population and closely associated with BCC risk (Table 2). In the Australian population, three RHC variants (D294H, R160W, and R151C) were those most closely associated with BCC risk (Box et al., 2001; Dwyer et al., 2004). In contrast, in our series, only R151C was associated with BCC, but the lack of association between the other RHC alleles – R142H, R160W, and D294H – and BCC risk could be due to their low allelic frequency, which would have made it necessary to study a larger population. These differences might be related to the specificity of the French population, which has a different ancestry and a darker complexion than the other populations studied, which were mainly of Celtic origin. In fact, compared to the Celtic population, RHC alleles had a global lower allelic frequency in both the French patients (11.2%) and the controls (6.6%).

The number of MC1R variants also appeared to modulate the risk of BCC, as the presence of at least two variants increased the risk by a factor 3 compared to the presence of only one MC1R variant (see Table 3). This genetic dosage effect had previously been reported for the association between MC1R and the risk of non-melanoma-skin cancer (Bastaens et al., 2001).

After stratification with known clinical risk factors for non-melanoma-skin cancer (i.e. skin type, eye and hair color, and
the presence of solar lentigines), the effect of MC1R variants on BCC risk persisted significantly (Table 4). This highlights the importance of MC1R variants as independent BCC risk factors, as previously noted in the Dutch and Australian populations (Bastiaens et al., 2001; Box et al., 2001).

MC1R variants had no influence on the age at diagnosis of BCC, the number of tumors or family history of BCC. This contrasts with BCC patients from The Netherlands, in whom the number of MC1R variants was significantly and independently associated with a higher number of skin tumors (Bastiaens et al., 2001). This suggests that additional genetic factors might account for the difference in BCC susceptibility in these populations. However, given the limited size of each subgroup, these data must be viewed with some caution and need to be confirmed in a larger set of patients.

In our population, the PTCH c.3944C/C genotype (corresponding to Pro/Pro) was significantly overrepresented in BCC patients, especially in the subgroup of patients with multiple BCCs (Table 5). The frequency of the c.3944C/C genotype has been shown to range from 30 to 65% across six normal human populations (Asplund et al., 2005). However, we do not yet know whether this variant is itself functional, or is in linkage disequilibrium with a functional polymorphism. No association with BCC risk could be demonstrated in a normal human populations (Asplund et al., 2005). However, this genotype was also more frequently present in US or Swedish patients with multiple BCC tumors (Asplund et al., 2005). Overall, these findings suggest that this genotype could be a risk factor for the development of BCC in the French population. However, some other studies do seem to show a potential protective effect on BCC of some PTCH haplotypes including this polymorphism in combination with other variants (intron 15, (G^10560+9) or exon 12, c.1686C) (Strange et al., 2004a,b). This means that further work is needed to clarify the role of this variant in genetic predisposition to BCC.

In conclusion, our study confirms the importance of MC1R variants among the BCC risk factors affecting the French population, which include both RHC and non-RHC alleles. Our work also suggests that PTCH gene exon 23 polymorphism c.3944C>T is involved in genetic susceptibility to BCC. These findings make it possible to improve the molecular identification of populations with a high risk of developing BCC, and this could be helpful in adapting appropriate preventive strategies.

MATERIALS AND METHODS

Study population

A prospective cohort of 126 patients with BCC and 151 controls were recruited between 1998 and 2004 in order to study environmental and genetic risk factors for BCC in the French population. The study population consisted of patients aged 24–91 years with histologically proven BCCs. All the participants were Caucasians recruited via the dermatology departments in four hospitals in Paris (France): the Ambroise Paré, Tarnier, Bichat Claude-Bernard, and Saint-Louis Hospitals, and all had given informed consent. The 126 patients were considered to be likely to have a genetic predisposition to BCC on the basis of at least one of the following criteria: familial BCC defined as the presence of a BCC in at least two first- or second-degree relatives (n = 26), multiple BCC (at least 2, n = 102), onset of BCC before the age of 40 years (n = 41), BCC associated with another cancer (n = 20) (colon, two cases; hematological malignancies, three cases; uterus, three cases; breast, five cases; skin squamous cell carcinoma, two cases; prostate, three cases; lung, one case; testis, one case). Since some patients had more than one criteria the total numbers of cases is above 126.

Patients who fulfilled the usual criteria for nevoid basal cell carcinoma syndrome (NBCCS) (Gorlin syndrome), or who were organ transplant recipients or suffered from xeroderma pigmentosum were excluded. None of the patients carried PTCH germline mutations.

The control group, recruited during the same period, was extended from one previously described (Matichard et al., 2004) and was composed of 151 individuals who had no personal or family history of skin cancer, who matched the patients in terms of age, sex, and geographical residence, and had been referred by the same departments and hospitals as the patients. The birthplace of the parents and grandparents were recorded to ensure that all the patients and control subjects were of Caucasian origin. All of the patients had been born in France, and most of their parents and grandparents (80%) were also all born in France. The remaining parents and grandparents (20%) had been born elsewhere in Europe.

The Hospital Medical Ethics Committee (CCPPRB) approved the study protocol. Informed consent was obtained from all the patients and control subjects enrolled in the study. The study was conducted in accordance with the Declaration of Helsinki Principles.

Participants were interviewed individually by a dermatologist, and a total skin examination was performed to collect data, which was recorded using a printed examination sheet. Information was recorded about solar lentigines, and eye color (classified as dark (brown or black) or light (blue, green/hazel, or gray)), and original hair color during early adulthood (classified using five categories: red, blond, light- or dark -brown, and black). Skin type was assessed according to the modified classification of Fitzpatrick as follows: always burns never tans (skin type I), always burns then tans (skin type II), always tans sometimes burns (skin type III), and always tans never burns (skin type IV) (Fitzpatrick, 1988). In addition, anatomical location of the tumor, age at diagnosis, and histopathological data were collected. The anatomical sites of BCC tumors were classified as follows: head and neck, trunk, or limbs.

The final series analyzed comprised 277 subjects: 126 BCC patients and 151 controls. The mean age and sex ratio did not differ in the two groups (Table 1). The BCC was of the superficial, nodular, infiltrating, and sclerodermiform histological subtype in respectively 88, 41, 30, and 17 BCC patients. Tumors involved one body site in 58.5% of patients (head and neck in 37%; trunk in 19%; limbs in 2.5%), two body sites in 24.5% of patients (head and neck and trunk, 14%; trunk and limbs in 8%; head and neck and limbs, 2.5%) and the three body sites (head and neck + trunk + limbs) in 17% of patients.

Detection of MC1R gene variants

Genomic DNA was isolated from peripheral blood leukocytes of all participants by routine methods (Miller et al., 1988). The MC1R
coding sequence was amplified by PCR with two overlapping pairs of primers, and sequenced as previously described (Matichard et al., 2004).

**Genotyping of PTCH variants**

The non-synonymous polymorphism in exon 23 of *PTCH*, c.3944C>T (L1315P, rs 357564) was genotyped using Taqman probe technology (Applied Biosystems, Foster City, CA, USA). Briefly, this product uses the S′ nuclease assay to discriminate between two alleles of a specific single nucleotide polymorphism for use in genotyping studies. Each assay is a 20× mix of forward primer, reverse primer, 6FAM™ dye – minor groove binder (MGB)-labeled probe, and VIC® dye – MGB-labeled probe. Each probe binds preferentially to one of the alleles. The detection of fluorescence was performed using a 7000 automate (Applied Biosystems).

A new polymorphism of *PTCH*, an 18 bp insertion localized 83 bp upstream from the donor splicing site of exon 2, was simply performed using a 7000 automate (Applied Biosystems).

**Statistical analyses**

Statistical analyses were performed using SAS software release 8.2 (SAS Institute, Cary, NC). \( \chi^2 \) analysis (plus Fisher’s exact test when necessary) was used to compare clinical and genetic characteristics between BCC patients and controls.

For clinical analysis, the BCC usual risk factors (skin type, solar lentigines, eye and hair colors) were compared between cases and controls.

For genetic analysis, only the MC1R polymorphisms considered to be functional were retained. These consisted of the following categories of polymorphisms:

1. MC1R variants that were unable to stimulate cAMP production as strongly as the wild-type receptor in response to α-MSH stimulation (Frandberg et al., 1998; Healy et al., 2001; Jimenez-Cervantes et al., 2001a, b; Schioth et al., 1999),
2. MC1R variants showing a decreased α-MSH binding affinity (Jimenez-Cervantes et al., 2001a; Ringholm et al., 2004),
3. MC1R variants previously shown to be associated with melanoma risk (Valverde et al., 1996; Palmer et al., 2000; Kennedy et al., 2001),
4. MC1R variants previously shown to be associated with the RHC phenotype (reviewed in Sturm, 2002),
5. MC1R variants predicted to be damaging by the Polyphen program and
6. an MC1R single nucleotide insertion producing a frameshift mutation that results in a prematurely terminated, non-functioning receptor.

The T95M, R163Q, V180L, and R223Q MC1R variants did not meet these criteria and were therefore considered to be wild type (Table 2). This conservative approach allowed us to include all the patients and controls in the analysis.

Firstly, univariate analyses were used to compare genetic risk factors (MC1R genotype) in BCC patients and controls.

Secondly, multiple logistic regression analysis was performed to take into account potential confounders among the clinical risk factors, such as skin type, eye and hair colors, and solar lentigines.

OR were calculated with their 95% confidence interval (CI). All significance levels reported were two sided and set at \( P<0.05 \).

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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