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 [1.34–4.8]). 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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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 is a seven-pass transmembrane G-protein coupled-receptor consisting of 317 amino acids, which is expressed in melanocytes and keratinocytes (Chhajlani, 1996). Stimulation of MC1R by α-melanocyte-stimulating hormone (α-MSH) leads to enhanced adenylate cyclase and cAMP activity, resulting in the synthesis of the black photoprotective

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Abbreviations: α-MSH, α-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 phaeomelanin (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 ($P \leqslant 0.0001$, odds ratio (OR) ranging from 2.98 to 4.91).

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)		
Men	55 (44)	67 (44)	0.86	NA
Mean age (years)	48 [24–91]	50 [19–100]		NA
Skin type				
III	33 (33)	62 (41)		Ref.[1]
IV	3 (3)	33 (22)		
I	11 (11)	6 (4)	**<0.0001**	**2.98 [1.77–5.01]**
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.
[1]OR 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.

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 (Total type–II variants, 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- or 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 2. Allelic frequencies of MC1R variants in BCC patients and control subjects

	BCC (*n*=252)	Controls (*n*=302)	*P*	OR [95% CI]
MC1R consensus	125 (49.6)	220		
MC1R variants type 1				
R163Q	10 (4)	8 (2.6)		
T95M	0	1		
V180L	0	1		
R223Q	1	0		
Total MC1R wt	**135 (53.6)**	**230 (76.5)**	Ref.	1
MC1R variants type 2				
V60L[1,2]	49 (19.4)	26 (8.6)	**0.0001**	**3.21 [1.91–5.38]**
S83P[2,3]	0	1	NA	NA
D84E[2–4]	0	2	NA	NA
V92M[5]	27 (10.7)	16 (5.3)	**0.001**	**2.87 [1.5–5.48]**
V92L[5]	0	1	NA	NA
N118K[3]	1	0	NA	NA
V122M[5]	0	1	NA	NA
R142H[1–3,5]	4 (1.6)	2 (0.7)	0.09[6]	3.40 [0.62–18.8]
R151C[1–4]	14 (5.5)	5 (1.6)	**0.0007**[6]	**5.11 [1.82–14.4]**
I155T[2,3]	2 (0.8)	2 (0.7)	NA	NA
R160W[1,2,4]	11 (4.4)	11 (3.6)	0.22	1.66 [0.7–3.93]
N281S[3]	1	0	NA	NA
D294H[1–5]	7 (2.7)	4 (1.3)	0.053[6]	2.98 [0.86–10.37]
R306H[3]	1	0	NA	NA
Ins 86[7]	1	1	NA	NA
Total variants type II	**117 (46.4)**	**72 (23.5)**	**<0.0001**	**2.77 [1.93–3.97]**

NA=not applicable; Ref.=reference.
Significant associations are shown in bold characters.
Type I variants may not modify *MC1R* function as these variants have not been previously associated with melanoma, and have not been tested in functional assays (see Statistical analyses in the Materials and Methods section). These variants are pooled with the *MC1R* consensus sequence, and considered as wild type in Statistical analyses.
Type II variants have been shown to result in diminished *MC1R* function, and/or associated with fair pigmentation characteristics (Sturm, 2002), and/or strongly associated with the risk of skin cancer in previous studies.
New variants that have not been previously identified are shown in italic characters. ORs are indicated with 95% CI.
[1]*MC1R* variants are 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 previously shown to be associated with the RHC phenotype (reviewed in (Sturm, 2002)).
[3]*MC1R* variants predicted to be damaging by the *Polyphen* program.
[4]*MC1R* variants previously shown to be associated with melanoma risk (Kennedy *et al.*, 2001; Palmer *et al.*, 2000; Valverde *et al.*, 1996).
[5]*MC1R* variants showing a decreased α-MSH-binding affinity (Jimenez-Cervantes *et al.*, 2001a).
[6]Fisher's exact test.
[7]*MC1R* single nucleotide insertion producing a frameshift mutation that results in a prematurely terminated, non-functioning receptor.

on sun-exposed sites (head and neck, limbs) or only on a less-exposed site (trunk) (data not shown).

Distribution of *PTCH* polymorphisms among cases and controls
The distribution of genotypes of the *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%, *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 ($P = 0.003$, OR 2.16 [1.3–3.6]), but not in that with a single BCC ($P = 0.70$). 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

Table 3. *MC1R* genotype in BCC patients and control subjects

	BCC cases (*n*=126)	Controls (*n*=151)	*P*-value	OR [95% CI]
(a) MC1R functional variants pooled				
Wt/Wt	41 (33)	89 (59)		Ref.
V/Wt	53 (42)	53 (35)	**0.004**	**2.17 [1.28–3.68]**
V/V	32 (25)	9 (6)	**<0.0001**	**7.72 [3.42–17.38]**
(b) RHC and non-RHC alleles separately				
Wt/Wt	41 (33)	89 (59)		Ref.
RHC/Wt	16 (13)	11 (7)	**0.005**[1]	**3.16 [1.35–7.40]**
RHC/RHC	2 (2)	3 (2)	0.33[1]	1.45 [0.23–9]
Non-RHC/Wt	37 (29)	41 (27)	**0.022**	**1.96 [1.1–3.48]**
Non-RHC/non-RHC	14 (11)	3 (2)	**<0.0001**[1]	**6.72 [2.76–37.2]**
RHC/non-RHC	15 (12)	4 (3)	**<0.0001**[1]	**4.83 [2.54–26]**

Ref.=reference; Wt=wild-type allele; V=variant alleles.
ORs compare Wt/V (heterozygotes) and V/V (two functional variants) to Wt/Wt. ORs are indicated with 95% CI.
[1]Fisher's exact test. Significant associations are shown in bold characters.
Association of MC1R variants with the risk (OR) of BCC

Table 4. Role of *MC1R* variants on BCC risk after stratification on clinical risk factors

Subjects characteristics	BCC (126)			Controls (151)				
MC1R genotype	**wt/wt**	**Wt/V**	**V/V**	**wt/wt**	**Wt/V**	**V/V**	**OR(1) V/wt *versus* wt/wt**	**OR(2) V/V *versus* wt/wt**
Skin type								
III	16	10	7	39	21	2	1.16 [0.45–2–97]	**8.63 [1.77–38.82]**
IV	1	1	1	17	14	2	1.2 [0.11–12.7]	8.5 [0.64–124.35]
I	3	3	5	1	3	2	0.33 [0.03–4.28]	0.83 [0.08–10.48]
II	14	27	13	30	17	3	**3.4 [1.42–8.13]**	**9.29 [2.4–35.2]**
Hair color								
Dark	7	9	1	29	16	2	2.33 [0.75–7.25]	2.07 [0.24–18.9]
Dark brown	8	10	5	34	23	3	1.85 [0.65–5.26]	**7.08 [1.51–33.03]**
Blond	4	10	7	6	4	0	3.75 [0.71–19.85]	NA
Light brown	11	10	7	17	11	1	1.4 [0.45–1.34]	**10.82 [1.85–74.82]**
Red	1	1	2	0	1	3	NA	NA
Eye color								
Dark	15	11	10	52	38	7	1 [0.42–2.34]	**4.95 [1.65–14.87]**
Light	18	30	13	35	16	2	**3.65 [1.6–8.32]**	**12.64 [2.82–55.05]**
Lentigines								
No	10	14	4	57	36	4	2.22 [0.9–5.43]	**5.7 [1.33–24.72]**
Yes	22	26	20	26	18	4	1.71 [0.75–3.88]	**5.91 [1.82–18.94]**

Wt=wild-type allele; V=variant alleles; NA=not applicable.
OR compare Wt/V (heterozygotes, OR no. 1) and V/V (two functional variants, OR no. 2) to Wt/Wt.
OR are indicated with 95% CI. Results are indicated by number of patients.
Significant associations are shown in bold characters.

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]).

Table 5. Association of *Patched* variant c. 3944C/T with BCC risk

c.3944C/T (rs357564)	BCC (*n*=125) (a)	Controls (*n*=151) (b)	*P*	OR[1]	MBCC (*n*=102)	*P*	0R[1]	SBCC (*n*=23)	*P*	OR[1]
Genotype frequencies										
Leu/Leu (c.3944 T/T)	15 (12)	24 (16)		Ref.	12 (12)		Ref.	3 (13)		Ref.
Pro/Leu (c.3944 C/T)	46 (37)	74 (49)			35 (34)			11 (48)		
Pro/Pro (c.3944 C/C)	64 (51)	53 (35)	**0.007**	**1.94 [1.2–3.1]**[1]	55 (54)	**0.003**	**2.16 [1.3–3.6]**[1]	9 (39)	0.7	1.19 [0.5–2.9][1]
Allelic frequency[2]	0.7	0.6	**0.016**		0.71	**0.008**		0.63	0.65	

HWE, Hardy–Weinberg equilibrium; MBCC=multiple BCC; Ref.=reference; SBCC=single BCC.
[1]Odd ratio was calculated by comparing the 3944C/C (Pro/Pro) genotype to the (3944C/T (Pro/Leu)+3944T/T (Leu/Leu)) genotypes.
[2]Allelic frequency of the major allele (C).
(a) *P*-value for HWE=0.55. (b) *P*-value for HWE=0.99.
Significant associations are shown in bold characters.

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 do 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.

Table 6. Multiple logistic regression analysis of BCC risk factors

Effect	*P*-value	OR	95% confidence limit
Skin type I or II	**0.03**	**2.11**	**1.08–4.14**
MC1R variants	**0.0044**	**2.53**	**1.34–4.8**
PTCH c.3944 CC (1315Pro/Pro)	0.15	1.6	0.83–3
Light hair color	**0.0021**	**2.9**	**1.47–5.7**
Solar lentigines	**<0.0001**	**4.67**	**2.46–8.89**

Logistic regression multiple analysis includes all clinical risk factors, *PTCH* genotype Pro/Pro and *MC1R* variants; ORs are indicated with 95% CI.
Significant associations are shown in bold characters.

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 (Bastiaens *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 relatively small series of 88 Swedish patients, despite the fact that the c.3944C/C genotype was more frequent in the BCC patients than in the controls (44 *vs* 35%) (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^{2560+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 BCCs 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 5′ 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 grove 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 genotyped by PCR amplification (primer sequence (5′→3′), forward CTG CGG CCC GGC TTT ATG AC, reverse GTG TGC GCT GGC GAA TAT CTC TAT C), and migration on a 2% agarose gel. PCR conditions included 35 denaturing cycles at 95°C for 30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 45 seconds.

Statistical analyses

Statistical analyses were performed using SAS software release 8.2 (SAS institute, Cary, NC). χ^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.

REFERENCES

Asplund A, Gustafsson AC, Wikonkal NM, Sela A, Leffell DJ, Kidd K *et al.* (2005) PTCH codon 1315 polymorphism and risk for nonmelanoma skin cancer. *Br J Dermatol* 152:868–73

Bastiaens MT, Huurne JA, Kielich C, Gruis NA, Westendorp RG, Vermeer BJ *et al.* (2001) Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. *Am J Hum Genet* 68:884–94

Box NF, Duffy DL, Irving RE, Russell A, Chen W, Griffyths LR *et al.* (2001) Melanocortin-1 receptor genotype is a risk factor for basal and squamous cell carcinoma. *J Invest Dermatol* 116:224–9

Chhajlani V (1996) Distribution of cDNA for melanocortin receptor subtypes in human tissues. *Biochem Mol Biol Int* 38:73–80

DePinho RA (2000) The age of cancer. *Nature* 408:248–54

Dwyer T, Stankovich JM, Blizzard L, FitzGerald LM, Dickinson JL, Reilly A *et al.* (2004) Does the addition of information on genotype improve prediction of the risk of melanoma and nonmelanoma skin cancer beyond that obtained from skin phenotype? *Am J Epidemiol* 159:826–33

Fitzpatrick TB (1988) The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 124:869–71

Frandberg PA, Doufexis M, Kapas S, Chhajlani V (1998) Human pigmentation phenotype: a point mutation generates nonfunctional MSH receptor. *Biochem Biophys Res Commun* 245:490–2

Gorlin RJ (2004) Nevoid basal cell carcinoma (Gorlin) syndrome. *Genet Med* 6:530–9

Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A *et al.* (1996) Mutations of the human homolog of Drosophila *PTCH* in the nevoid basal cell carcinoma syndrome. *Cell* 85:841–51

Han J, Colditz GA, Samson LD, Hunter DJ (2004) Polymorphisms in DNA double-strand break repair genes and skin cancer risk. *Cancer Res* 64:3009–13

Healy E, Jordan SA, Budd PS, Suffolk R, Rees JL, Jackson IJ (2001) Functional variation of MC1R alleles from red-haired individuals. *Hum Mol Genet* 10:2397–402

Jimenez-Cervantes C, Germer S, Gonzalez P, Sanchez J, Sanchez CO, Garcia-Borron JC (2001a) Thr40 and Met122 are new partial loss-of-function natural mutations of the human melanocortin 1 receptor. *FEBS Lett* 508:44–8

Jimenez-Cervantes C, Olivares C, Gonzalez P, Morandini R, Ghanem G, Garcia-Borron JC (2001b) The Pro162 variant is a loss-of-function mutation of the human melanocortin 1 receptor gene. *J Invest Dermatol* 117:156–8

Jones FI, Ramachandran S, Lear J, Smith A, Bowers B, Ollier WE *et al.* (1999) The melanocyte stimulating hormone receptor polymorphism: association of the V92M and A294H alleles with basal cell carcinoma. *Clin Chim Acta* 282:125–34

Kennedy C, ter Huurne J, Berkhout M, Gruis N, Bastiaens M, Bergman W *et al.* (2001) Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. *J Invest Dermatol* 117:294–300

Kricker A, Armstrong BK, English DR (1994) Sun exposure and non-melanocytic skin cancer. *Cancer Causes Control* 5:367–92

Lear JT, Heagerty AH, Smith A, Bowers B, Payne CR, Smith CA *et al.* (1996) Multiple cutaneous basal cell carcinomas: glutathione *S*-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. *Carcinogenesis* 17: 1891–6

Lovatt T, Alldersea J, Lear JT, Hoban PR, Ramachandran S, Fryer AA *et al.* (2005) Polymorphism in the nuclear excision repair gene ERCC2/XPD: association between an exon 6-exon 10 haplotype and susceptibility to cutaneous basal cell carcinoma. *Hum Mutat* 25:353–9

Magnaldo T, Sarasin A (2004) Xeroderma pigmentosum: from symptoms and genetics to gene-based skin therapy. *Cells Tissues Organs* 177:189–98

Matichard E, Verpillat P, Meziani R, Gerard B, Descamps V, Legroux E *et al.* (2004) Melanocortin 1 receptor (MC1R) gene variants may increase the risk of melanoma in France independently of clinical risk factors and UV exposure. *J Med Genet* 41:e13

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl Acids Res* 16:1215

Naldi L, DiLandro A, D'Avanzo B, Parazzini F (2000) Host-related and environmental risk factors for cutaneous basal cell carcinoma: evidence from an Italian case–control study. *J Am Acad Dermatol* 42:446–52

Palmer JS, Duffy DL, Box NF, Aitken JF, O'Gorman LE, Green AC *et al.* (2000) Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? *Am J Hum Genet* 66:176–86

Ramachandran S, Fryer AA, Smith AG, Lear JT, Bowers B, Hartland AJ *et al.* (2001) Basal cell carcinomas: association of allelic variants with a high-risk subgroup of patients with the multiple presentation phenotype. *Pharmacogenetics* 11:247–54

Rees JL (2004) The genetics of sun sensitivity in humans. *Am J Hum Genet* 75:739–51

Ringholm A, Klovins J, Rudzish R, Phillips S, Rees JL, Schioth HB (2004) Pharmacological characterization of loss of function mutations of the human melanocortin 1 receptor that are associated with red hair. *J Invest Dermatol* 123:917–23

Schioth HB, Phillips SR, Rudzish R, Birch-Machin MA, Wikberg JE, Rees JL (1999) Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. *Biochem Biophys Res Commun* 260:488–91

Strange RC, El-Genidy N, Ramachandran S, Lovatt TJ, Fryer AA, Smith AG *et al.* (2004a) PTCH polymorphism is associated with the rate of increase in basal cell carcinoma numbers during follow-up: preliminary data on the influence of an exon 12-exon 23 haplotype. *Environ Mol Mutagen* 44:469–76

Strange RC, El-Genidy N, Ramachandran S, Lovatt TJ, Fryer AA, Smith AG *et al.* (2004b) Susceptibility to basal cell carcinoma: associations with PTCH polymorphisms. *Ann Hum Genet* 68:536–45

Sturm RA (2002) Skin colour and skin cancer – MC1R, the genetic link. *Melanoma Res* 12:405–16

Sturm RA, Duffy DL, Box NF, Chen W, Smit DJ, Brown DL *et al.* (2003a) The role of melanocortin-1 receptor polymorphism in skin cancer risk phenotypes. *Pigment Cell Res* 16:266–72

Sturm RA, Duffy DL, Box NF, Newton RA, Shepherd AG, Chen W *et al.* (2003b) Genetic association and cellular function of MC1R variant alleles in human pigmentation. *Ann NY Acad Sci* 994: 348–58

Valverde P, Healy E, Sikkink S, Haldane F, Thody AJ, Carothers A *et al.* (1996) The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. *Hum Mol Genet* 5:1663–6

Wong CS, Strange RC, Lear JT (2003) Basal cell carcinoma. *BMJ* 327: 794–8