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RESEARCH ARTICLE

Variants of the *MATP/SLC45A2* Gene Are Protective for Melanoma in the French Population

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In this study, we investigated whether variants in three key pigmentation genes—*MC1R*, *MATP/SLC45A2*, and *OCA2*—were involved in melanoma predisposition. A cohort comprising 1,019 melanoma patients (MelanCohort) and 1,466 Caucasian controls without skin cancers were studied. A total of 10 polymorphisms, including five functional *MC1R* alleles (p.Asp84Glu, p.Arg142His, p.Arg151Cys, p.Arg160Trp, and p.Asp294His), two nonsynonymous *SLC45A2* variants (p.Phe374Leu and p.Glu272Lys), and three intronic *OCA2* variants previously shown to be strongly associated with eye color (rs7495174 T>C, rs4778241 G>T, and rs4778138 T>C) were genotyped. As expected, *MC1R* variants were closely associated with melanoma risk (P value $<2.20.10^{-16}$; odds ratio [OR] = 2.29 [95% confidence interval, CI = 1.85–2.82 and OR = 3.3 [95% CI = 2.00–5.45], for the presence of one or two variants, respectively). Interestingly, the *SLC45A2* variant p.Phe374Leu was significantly and strongly protective for melanoma (*P*-value = $2.12.10^{-15}$; OR = 0.35 [95% CI = 0.26–0.46] and OR = 0.32 [95% CI = 0.24–0.43], considering the genotypes Phe/Leu and Leu/Leu, respectively). *MC1R* and *SLC45A2* variants had additive effects on melanoma risk, and after adjusting for pigmentation characteristics, the risk was persistent, even though both genes had a strong impact on pigmentation. Future studies may show whether genetic information could provide a useful complement to physical examination in predicting melanoma risk. Hum Mutat 29(9), 1154–1160, 2008.

KEY WORDS: cancer; melanoma; MC1R; SLC45A2; pigmentation

INTRODUCTION

Major advances have been made in the identification of genetic factors that predispose to melanoma. A positive family history of the disease is among the most clearly established risk factors for melanoma [Greene, 1999]. Two highly penetrant melanoma-predisposing genes have been identified to date, *CDKN2A* and *CDK4*, that are mainly involved in predisposition to familial melanoma and to multiple sporadic melanoma [Hussussian et al., 1994; Kamb et al., 1994; Zuo et al., 1996]. Recent linkage studies have also identified additional major susceptibility loci on chromosomes 1p22 [Gillanders et al., 2003] and 9q21 [Jonsson et al., 2005].

There is also increasing evidence of a polygenic inheritance of melanoma, and identification of other gene variants that contribute to melanoma risk is in progress. These include a key pigmentation gene, *MC1R* (MIM# 155555), which has been shown to act both as a low penetrance melanoma gene [Debniak et al., 2006; Healy, 2004; Kennedy et al., 2001; Landi et al., 2005; Matichard et al., 2004; Palmer et al., 2000; Stratigos et al., 2006] and as a genetic modifier of melanoma risk in individuals carrying

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CDKN2A mutations [Box et al., 2001; Chaudru et al., 2005; van Der Velden et al., 2001].

The *MC1R* gene is localized at 16q, and encodes a seven-pass transmembrane G-protein coupled-receptor, which is expressed in several cell types including melanocytes. Stimulation of *MC1R* by the α-melanocyte-stimulating hormone (α-MSH) results in the synthesis of the black photoprotective pigment eumelanin. *MC1R* is highly polymorphic in Caucasian populations, and numerous *MC1R* variants have been demonstrated to result in a loss of function, reducing either cAMP production or α-MSH binding affinity [Frandberg et al., 1998; Healy et al., 2001; Jimenez-Cervantes et al., 2001a, 2001b; Ringholm et al., 2004; Schioth et al., 1999]. Five *MC1R* variant alleles (p.Asp84Glu, p.Arg142His, p.Arg151Cys, p.Arg160Trp, and p.Asp294His) have been shown to be strongly associated with a phenotype characterized by red hair, fair skin, freckles, and sun sensitivity, and are known as the RHC or R variants (RHC, for red hair color). In addition, it is now clear that RHC variants play an important role in determining the risks of melanoma and nonmelanoma-skin cancer (NMSC) [reviewed in Sturm et al., 2003]. Another key pigmentation gene is the membrane-associated transporter protein gene (*MATP*; MIM# 606202). Originally identified as an antigen in human melanoma and known as AIM1, it has recently been renamed solute carrier family 45 member 2 (*SLC45A2*) [Harada et al., 2001]. The *MATP/SLC45A2* gene is located on chromosome 5p, comprises seven exons spanning 40 kb, and encodes a 530–amino acid protein presumably located in the melanosome membrane [Newton et al., 2001]. *SLC45A2* exhibits structural homology to plant sucrose-proton symporters and probably directs the traffic of melanosomal proteins and other substances to the melanosomes [Costin et al., 2003]. *SLC45A2* mutations cause pigmentation variation in the medaka [Fukamachi et al., 2001], mouse [Newton et al., 2001; Du and Fisher, 2002], horse [Mariat et al., 2003], chicken, and Japanese quail [Gunnarsson et al., 2007]. In humans, pathogenic mutations in *MATP/SLC45A2* lead to type IV oculocutaneous albinism. *SLC45A2* mutations disrupt tyrosinase processing and trafficking at the post-Golgi level [Hearing, 2005; Kushimoto et al., 2003].

Two human *SLC45A2* polymorphisms, p.Glu272Lys and p.Phe374Leu, display differing population frequency distributions. Interestingly, these variants have been shown to be significantly associated with dark hair, skin, and eye pigmentation in a Caucasian population [Graf et al., 2005].

A third gene known to play a crucial role in human pigmentation is *OCA2* (*P* gene; MIM# 611409) [Ancans et al., 2003; Sturm et al., 2003], which is the gene responsible for the most prevalent type of tyrosinase-positive oculocutaneous albinism (OCA, type II) [Lee et al., 1994]. The *OCA2* gene maps to chromosome 15q11.2–q12, is highly polymorphic [Lee et al., 1995], and encodes an 838–amino acid melanosomal protein resembling a channel or a transporter [Gardner et al., 1992; Ramsay et al., 1992; Rinchik et al., 1993; Rosemblat et al., 1998; Staleva et al., 2002]. *OCA2* plays an important role in controlling the eumelanin content of melanocytes [Hirobe et al., 2002; Lamoreux et al., 1995; Orlow and Brilliant, 1999; Ozeki et al., 1995; Tamate et al., 1989] via the processing of tyrosinase and the regulation of the melanosomal pH [Chen et al., 2002; Toyofuku et al., 2002]. It has recently been shown that three *OCA2* intronic variants (rs7495174, rs4778241, and rs4778138) were closely associated with normal phenotypic variations in human eye color [Duffy et al., 2007]. In addition, more recently, a common founder mutation in an *OCA2*-inhibiting regulatory element localized within the *HERC2* gene (MIM#: 605837) has been identified as strongly associated with blue eye color in humans [Sturm et al., 2008; Kayser et al., 2008; Eiberg et al., 2008].

As pigmentation characteristics and melanoma risk are closely correlated, we investigated the impact of three of these pigmentation genes (*MC1R*, *SLC45A2*, and *OCA2*) on melanoma risk by genotyping 10 SNPs in a large case–control study from France (1,019 patients, 1,466 Caucasian controls without skin cancer from the same area). In addition to the well-known association of *MC1R* variants with melanoma risk, we found evidence that *SLC45A2* variants have a strong protective effect for melanoma that persisted in multivariate analysis including clinical and genetic melanoma risk factors.

MATERIALS AND METHODS

Materials

Study population. A total of 1,019 melanoma patients and 1,466 control subjects, all of Caucasian origin, were prospectively included in the study between 2003 and 2006. Melanoma patients were recruited between 2003 and 2006 from the MelanCohort, a prospective melanoma cohort including all melanoma patients from the Dermatology Departments of all the University-affiliated hospitals of Paris (Bichat, Percy, Ambroise Paré, Henri Mondor, Cochin, and Saint-Louis Hospitals). The inclusion criteria of the studied population are described in the Supplementary Methods (available online at http://www.interscience.wiley.com/jpages/1059-7794/suppmat).

A control group was composed of 1,466 Caucasian individuals (subjects who were from African or Asian descent were excluded), with no personal history of skin cancer (as assessed by answering to a preprint questionnaire). The Caucasian control subjects were recruited in the same demographic area among blood donors (region of Paris), and data on pigmentation characteristics were collected for 220 of these subjects after physical examination by dermatologists (see below).

Median age and sex ratio were not statistically different between the groups of patients and controls.

The local Medical Ethics Committee (CCPPRB) approved the study protocol. Informed consent was obtained from all the patients and control subjects enrolled in the study. After informed consent, genomic DNA was isolated from peripheral blood leukocytes of all participants using the QIAamp Blood Mini Kit (QIAgen GmbH, Hilden, Germany).

Collecting data about risk factors for melanoma. Data on pigmentation characteristics (skin type, eye and hair colors, nevi count, and presence of solar lentigines) were collected for all patients and 220 control subjects. A standardized personal interview and a whole-body skin examination were performed by dermatologists from Bichat and Saint Louis hospitals, and were reported using a standard examination report form (see Supplementary Methods).

Genotyping MC1R, SLC45A2, and OCA2 Variants

MC1R and *SLC45A2* cDNA reference sequences with GenBank accession Nos. NM_002386.2 and NM_016180.3 were used. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

The *MC1R* polymorphisms retained for genetic analysis were those associated with the red hair color phenotype (RHC alleles), and included c.252C>A p.Asp84Glu, c.425G>A p.Arg142His,

c.451T>C p.Arg151Cys, c.478C>T p.Arg160Trp, and c.880G>C p.Asp294His [Duffy et al., 2004; Flanagan et al., 2000; Rees, 2004].

The two *SLC45A2* variants studied were nonsynonymous SNPs, c.814G>A p.Glu272Lys and c.1122C>G p.Phe374Leu (NCBI dbSNP rs26722 and rs16891982, dbSNP; www.ncbi.nlm.nih.gov/SNP).

The *OCA2* variants studied were the three intronic SNPs described above (rs7495174 T>C, rs4778241 G>T, and rs4778138 T>C) [Duffy et al., 2007].

All SNPs were genotyped using the KBiosciences PCR SNP genotyping system (KASPAR SNP Genotyping System KBiosciences, Hoddesdon Herts, UK), which is a homogeneous fluorescent genotyping system, using a unique form of allele-specific PCR allowing allelic discrimination. Validation of the genotyping data was then performed by two other methods (see Supplementary Methods).

MC1R variants genotyping was successfully completed in 838 patients and 1,067 controls. Both *SLC45A2* variants were genotyped efficiently in 965 patients and 1,426 controls. *OCA2* variants rs7495174, rs4778241, and rs4778138 were genotyped in 970, 828, and 988 patients and 1377, 1341, and 1363 controls, respectively.

Statistical Analysis

The main part of the statistical analysis was performed using the R computer package (version 2.6.0) (Boston, MA). The level of significance for all the tests was set to a level corresponding to a type-I error-rate of $\alpha = 5\%$. Considering the number of tests performed, and the high levels of the associations, most of them would remain significant if a correction for multiple testing was applied. All the odds ratios (ORs) are reported with their 95% confidence interval (CI).

Association of genetic factors with melanoma. Conformity to Hardy-Weinberg equilibrium was tested in controls using a classical one degree-of-freedom, chi-square test. Individual association analyses of genetic factors with melanoma (*MC1R*, *SLC45A2*, and *OCA2* variants) were performed by comparing cases and controls using a Fisher's exact test of genotypes (coded as 0, 1, and 2, with 0 being the most frequent genotype). The corresponding ORs were assessed using a standard logistic regression analysis on the data with, for each polymorphism, the reference taken as being the most frequent genotype (0). In addition, an analysis of haplotype diversity was carried out on each gene by the expectation-maximization (EM) algorithm using the Arlequin software (version 3.01) [Schneider et al., 2000]. Linkage disequilibrium (LD) between pairs of polymorphic sites was measured in controls using the common statistics D, D' [Lewontin, 1964] and r [Hill and Robertson, 1968]). Finally, gene-gene interactions were evaluated using logistic regression.

Association of clinical factors with melanoma. Individual analyses of the usual clinical factors (skin color: fair and medium vs. dark, skin type: I-II vs. III-IV, hair color: red-blond-light brown vs. dark brown-black, eye color: light vs. black, nevus count: ≤ 50 vs. ≥ 51, ephelides and dorsal lentigines: presence vs. absence) were also performed by comparing all the cases and 220 controls by a Fisher's exact test of genotypes. The corresponding ORs were once again assessed using a logistic regression analysis on the data.

Association of genetic factors with clinical factors. Since the three genes we were investigating all act on pigmentation, individual association analyses of genetic factors for each clinical factor were also performed and adjusted for the case-control status. The corresponding ORs were assessed via logistic regressions.

Integration of genetic and clinical factors. Because pigmentation characteristics are associated with the three pigmentation genes and with melanoma risk, they can constitute potential confounders. In this part of the analyses, we used a logistic model based on genetic and clinical risk factors to check whether the genetic associations with melanoma were still found in the presence of pigmentation characteristics.

RESULTS

Association of Genetic Factors With Melanoma

In the controls, the genotype frequencies for all the polymorphisms tested were in Hardy-Weinberg equilibrium. In the subgroup of controls whose pigmentation characteristics were collected (Supplementary Table S1), genotyping data were representative of the whole set of controls.

As shown in Table 1, the presence of RHC *MC1R* variants was significantly associated with melanoma risk. The risk of melanoma increased seriously with the number of RHC alleles from OR = 2.29 with one variant allele, to OR = 3.3 with two variant alleles, the effect of these polymorphisms on the disease therefore being very close to additive. Supplementary Table S2 shows the results of the association study for all five RHC polymorphisms. With the exception of p.Arg142His, all were significantly associated with melanoma risk.

As shown in Table 2, both *SLC45A2* variants (p.Phe374Leu and p.Glu272Lys) were significantly and strongly protective for melanoma (P value $= 2.12.10^{-15}$ and $1.10.10^{-6}$, respectively, OR close to 0.3 for both variants).

The *SLC45A2* 374Phe allelic frequency in controls was 0.90, which was the same as that previously reported in the general French population [Yuasa et al., 2006], whereas it was 0.96 in patients (P value $= 1.30.10^{-11}$).

Haplotype analysis confirmed the protective effect of *SLC45A2* variants for melanoma (Fisher's exact test applied to haplotype frequencies P value $= 3.67.10^{-15}$, Supplementary Table S3, Supplementary Data). The 272Lys374Leu and 272Glu374Leu haplotypes were significantly more frequent in controls (a difference which was due to the p.Phe374Leu variant as indicated in Supplementary Table S3), and were therefore considered to have a protective effect for melanoma. A similar analysis of diplotypes indicates that two diplotypes present in up to 17.76% of the controls were also protective (Supplementary Table S3).

As we observed an almost complete LD between the two polymorphisms ($D = 0.036$, $D' = 0.92$ and $r = 0.59$), only the *SLC45A2* Phe374Leu was retained in the subsequent statistical analyses.

Finally, we sequenced the entire coding sequence (seven exons) of this gene in 48 melanoma patients; no other nonsynonymous variant or pathogenic mutation of *SLC45A2* was identified.

TABLE 1. **Association of *MC1R* Variants With Melanoma**

MC1R genotypes	Patients (%)	Controls (%)	P value*	OR [95% CI]
Total	**838**	**1,067**		
0/0	**510 (60.9)**	**841 (79.9)**		**Reference**
1/0	**280 (33.4)**	**202 (18.9)**	**$< 2.20.10^{-16}$**	**2.29 [1.85–2.82]**
1/1	**48 (5.7)**	**24 (2.25)**		**3.3 [2–5.45]**

*P value was calculated by applying Fisher's exact test to the genotype counts in cases and controls.

TABLE 2. Association of *SLC45A2* and *OCA2* Variants With Melanoma[†]

SNP identity	Allele	Genotypes	Patients (%)	Controls (%)	P value*	OR [95% CI]
SLC45A2-rs26722	c.814G>A p.Glu272Lys	Total	968	1,427		
		GG	933 (96.4)	1,305 (91.4)		Reference
		GA	33 (3.4)	1 (8.3)	$1.10.10^{-6}$	0.39 [0.26–0.58]
		AA	2 (0.2)	3 (0.2)		0.93 [0.16–5.59]
		MAF	1.9%	4.4%		
SLC45A2-rs16891982	c.1122C>G p.Phe374Leu	Total	965	1426		
		GG	895 (92.7)	1,160 (81.3)		Reference
		GC	65 (6.7)	246 (17.3)	$2.12.10^{-15}$	0.34 [0.26–0.46]
		CC	5 (0.5)	20 (1.4)		0.32 [0.24–0.43]
		MAF	3.9%	10.1%		
OCA2-rs7495174	T>C	Total	988	1,377		
		TT	804 (81.4)	1,072 (77.9)		Reference
		CT	168 (17.0)	279 (20.3)	0.11	0.80 [0.65–0.99]
		CC	16 (1.6)	26 (1.9)		0.82 [0.44–1.54]
		MAF	10.1%	12.1%		
OCA2-rs4778241	G>T	Total	828	1341		
		GG	479 (57.9)	750 (55.9)		Reference
		TG	299 (36.1)	508 (37.9)	0.68	0.92 [0.77–1.11]
		TT	50 (6)	83 (6.2)		0.94 [0.65–1.36]
		MAF	24.1%	25.1%		
OCA2-rs4778138	T>C	Total	970	1363		
		TT	662 (68.2)	844 (61.9)		Reference
		CT	271 (27.9)	457 (33.5)	$6.96.10^{-3}$	0.76 [0.63–0.91]
		CC	37 (3.8)	62 (4.6)		0.76 [0.50–1.16]
		MAF	0.18	21.4%		

[†]*SLC45A2* cDNA reference sequences with GenBank accession No. NM.016180.3 were used. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.
*P value was calculated by applying Fisher's exact test to the genotype counts in cases and controls.
MAF, minor allele frequency.

TABLE 3. Calculation of the Combined ORs for the *MC1R* and *SLC45A2* p.Phe374Leu Genotypes*

MC1R genotypes	*SLC45A2* p.Leu374Phe genotypes	Patients (%)	Controls (%)	OR [95% CI]
	Total	792	868	
0/0	GG	444 (56.1)	561 (64.6)	Reference
0/0	GC	32 (4)	118 (13.6)	0.34 [0.23–0.51]
0/0	CC	3 (0.4)	13 (1.5)	0.29 [0.09–0.96]
0/1	GG	249 (31.4)	130 (15)	2.42 [1.89–3.09]
0/1	GC	20 (2.5)	30 (3.5)	0.84 [0.47–1.49]
0/1	CC	2 (0.3)	3 (0.3)	1.26 [0.22–7.19]
1/1	GG	39 (4.9)	11 (1.3)	4.48 [2.29–8.74]
1/1	GC	3 (0.4)	2 (0.2)	1.89 [0.38–1.52]
1/1	CC	0	0	na

*The most frequent combination of genotypes (absence of RHC variants for *MC1R* and the GG genotype for *SLC45A2* p.Phe374Leu, the GG genotype) is taken as the reference for OR computation.
na, not applicable.

Only one *OCA2* SNP (OCA2-rs4778138) was weakly associated with melanoma (P value = $6.93.10^{-3}$; OR = 0.80 [95% CI = 0.69–0.92], Table 2), but the association was no longer significant after correcting for multiple testing. Analyses of the haplotypes and diplotypes inferred from the three *OCA2* SNPs did not indicate any further significant association of this gene with melanoma, although the TGT haplotype and diplotype previously shown to be strongly associated with light eye color were more frequently observed in melanoma patients than in controls (Supplementary Table S3).

Table 3 illustrates the various possible "at risk" or "protective" combinations of the *MC1R* and *SLC45A2* genotypes, indicating an additive effect of both genes on melanoma risk.

Finally, a study of the potential interaction between the presence of the *MC1R* variants and each of the *SLC45A2* or *OCA2* variants was performed, but no significant gene-gene interaction could be identified.

Association of Clinical Factors With Melanoma

A case-control study of the main clinical risk factors was performed between all the melanoma patients and the 220 controls whose pigmentation characteristics had been collected. As shown in Table 4, the greatest risk factors were having a nevus count $\geq$50 (OR = 5.91), light eye color (OR = 2.55), skin types I or II (OR = 2.35), fair hair color (OR = 2.18), and solar lentigines (OR = 3.19).

Association of Genetic Factors With Clinical Factors

Since the three genes we were investigating all act on pigmentation, individual association analyses of genetic factors on the main clinical risk factors were also performed and adjusted to the case-control status.

The most significant association was that between *OCA2* and light eye color (P value $<2.20.10^{-16}$ for each of the three *OCA2* SNPs), which confirms the recently demonstrated association between these SNPs and eye color [Duffy et al., 2007]. The TGT haplotype was also strongly associated with light eye color (P value $<2.20.10^{-16}$; OR = 5.85 [95% CI = 8.10–4.23]). All three *OCA2* variants and the TGT haplotype were also strongly associated with light hair color (red, blond or light brown) (P value $<1.00.10^{-6}$; OR = 2.27 [95% CI = 2.95–1.75]).

SLC45A2 p.Phe374Leu was shown to be associated with dark eye color (P value = $1.47.10^{-8}$; OR = 3.55 [95% CI = 2.33–5.56]), dark hair color (dark brown or black) (P value = $5.26.10^{-9}$;

TABLE 4. Associations of Clinical Risk Factors With Melanoma

Clinical factors	P value*	OR [95% CI]
Skin type (I-II vs. III-IV)	$4.69.10^{-8}$	2.35 [1.71–3.25]
Hair color (blond, light brown, red vs. dark brown, black)	$4.66.10^{-7}$	2.18 [1.59–3.01]
Eye color (light vs. dark)	$2.20.10^{-9}$	2.55 [1.87–3.48]
Nevi count (>50 vs. ≤50)	$2.65.10^{-14}$	5.91 [3.38–10.36]
Solar lentigines (presence vs. absence)	$4.52.10^{-10}$	3.19 [2.17–4.71]

*The P value was calculated by applying Fisher's exact test to each clinical risk factor in patients and controls.

TABLE 5. Regression Logistic Model Integrating Clinical and Genetic Melanoma Risk Factors

Melanoma risk factors	P value*	OR [95% CI]
Skin type (I-II vs. III-IV)	$4.42.10^{-2}$	1.49 [1.01–2.19]
Hair color (blond, light brown, red vs. dark brown, black)	0.14	1.35 [0.90–2.03]
Eye color (light vs. dark)	$1.16.10^{-4}$	2.16 [1.46–3.21]
Nevi count (>50 vs. ≤50)	$1.34.10^{-7}$	5.43 [2.89–10.18]
MC1R variants (presence vs. absence)	$8.01.10^{-5}$	2.23 [1.50–3.32]
SLC45A2 p.Phe374Leu genotype (CC+GC vs. GG)	$4.16.10^{-3}$	0.49 [0.30–0.79]

*The P value was calculated by applying Fisher's exact test to each clinical risk factor in patients and controls.

OR = 3.57 [95% CI = 2.32–5.56]) and skin type III-IV (P value = $1.95.10^{-10}$; OR = 4.55 [95% CI = 2.86–7.14]).

Finally, *MC1R* variants showed a significant association with light hair color (P value = $6.83.10^{-9}$; OR = 2.02 [95% CI = 2.57–1.59]) and skin type I-II (P value = $1.71.10^{-12}$; OR = 2.37 [95% CI = 3.00–1.86]).

Remarkably, the nevus count, which was the most important clinical predictor of melanoma in our study, was not statistically associated with any of these pigmentation genes.

Integration of Genetic and Clinical Risk Factors

Interestingly, the association of *MC1R* variants with melanoma and the protective effect of *SLC45A2* p.Phe374Leu remained highly significant in a logistic model integrating pigmentation characteristics (Table 5).

We also calculated the cumulative ORs resulting from combining these factors. According to the model, the OR of individuals with a nevus count ≥50, skin type I-II, light eye color, at least one RHC allele for *MC1R*, and the most frequent *SLC45A2* p.Phe374Leu genotypes (GG) was 40 times higher than the one of subjects with a nevus count <50, no RHC allele, skin type III-IV, dark eye color, and one of the protective *SLC45A2* genotypes (CG or CC).

DISCUSSION

Human hair, skin, and eye color are all complex physical traits and probably result from interactions between more than 120 genes (International Albinism Centre Albinism Database; http://albinismdb.med.umn.edu). Combined with environmental influences, genetic polymorphisms that affect the production and deposition of melanin, as well as the formation/maturation of the melanosome, could therefore modulate the individual skin cancer risk. In this study, we investigated the role of polymorphism of three key pigmentation genes (*MC1R*, *SLC45A2*, and *OCA2*) on melanoma risk in a large sample of patients and controls.

The role of *MC1R* on melanoma risk has now been analyzed in numerous populations (Australian, Dutch, French, Greek, Italian, and Polish). The results of these studies are consistent, showing an increase in melanoma risk (OR>2) in individuals carrying one *MC1R* variant, the risk rising when two alleles are present [Debniak et al., 2006; Kennedy et al., 2001; Landi et al., 2005; Matichard et al., 2004; Palmer et al., 2000; Stratigos et al., 2006]. Although their allelic frequencies vary across the different populations studied, the RHC or R variants have been shown to be the most strongly associated with melanoma risk [Matichard et al., 2004; Stratigos et al., 2006]. However, the impact of other *MC1R* variants (*r* variants) on melanoma risk is still debated [Gerstenblith et al., 2007]. Our data confirm previous findings in a large European population, and establish clearly the role of *MC1R* RHC variants on melanoma risk that is independent of pigmentation characteristics (see Table 1, Supplementary Table S2, and Table 5).

In addition, we show here for the first time that *SLC45A2* polymorphisms are associated with a strong protective effect for melanoma that persisted after correcting for multiple-testing (Table 2 and Supplementary Table S3). Sequence variation analyses of the *SLC45A2* gene have been extensively performed across different populations [Nakayama et al., 2002; Soejima and Koda, 2007; Soejima et al., 2006; Yuasa et al., 2004, 2006]. A positive selection has been demonstrated in Europeans that favors an haplotype containing the *SLC45A2* 374Phe allele [Soejima and Koda, 2007; Soejima et al., 2006]. The 374Leu ancestral allele was shown to be associated with dark pigmentation (skin, eye, and hair colors) in a population of European ancestry [Graf et al., 2005]. Therefore, it has been speculated that the *SLC45A2* 374Phe allele—which is associated with light hair, skin, and eye pigmentation—is advantageous for vitamin D synthesis at higher latitudes.

An effect of this variant on human pigmentation has also been demonstrated by reflectance spectrophotometry in African-Americans, the *SLC45A2* 374Leu allele being estimated to have an effect size per allele of 15 melanin units [Norton et al., 2007]. In our study, this allele—associated with a higher melanin concentration—was clearly protective for melanoma (Table 2 and Supplementary Table S3).

We also observed that the protective effect of this *SLC45A2* variant persisted after adjusting for pigmentation characteristics (Table 5) although it was strongly associated with dark pigmentation (i.e., skin type III-IV, dark brown and black hair, and dark eye color). This suggests that with regard to melanoma risk, information related to the *SLC45A2* p.Phe374Leu genotype is not redundant with that for pigmentation characteristics.

The second *SLC45A2* variant p.Glu272Lys was also strongly protective for melanoma (Table 2). As p.Glu272Lys and p.Leu374Phe are in close linkage disequilibrium, we considered that this association was contingent on that of the p.Leu374Phe variant. However, promoter polymorphisms in the *SLC45A2* gene have also recently been shown to be associated with normal human skin color variation [Graf et al., 2007], and it is possible that other *SLC45A2* variants may also influence melanoma risk.

The third pigmentation gene studied was *OCA2*, a gene tightly linked to eye color. We showed recently that a combination of two *OCA2* SNPs was associated with melanoma risk in a limited case-control study from France [Jannot et al., 2005]. Interestingly, one of these SNPs (A776A, rs1800419) was found to be tightly associated with eye color in a twins study from Australia [Duffy et al., 2007]. In this larger study, we showed that another *OCA2* SNP known to be strongly associated with eye color (OCA2 rs4778138) was also weakly associated with melanoma (Table 2). Yet, this association was no longer found after adjusting for eye

color or correcting for multiple testing, which means that this SNP is not a strong independent genetic risk factor for melanoma in our population.

In conclusion, in this large case-control study, we have demonstrated the strong influence of the variation of two pigmentation genes on melanoma risk, which persisted in statistical models integrating pigmentation characteristics as potential cofounders. Future studies might seek to answer whether genetic information could provide a useful complement to physical examination in predicting melanoma risk.

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