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MC1R, *SLC45A2* and *TYR* genetic variants involved in melanoma susceptibility in Southern European populations: Results from a Meta-analysis

Maider Ibarrola-Villava [a,j], Hui-Han Hu [b,c,j], Mickaël Guedj [c], Lara P. Fernandez [d], Vincent Descamps [c,e], Nicole Basset-Seguin [c,f], Martine Bagot [c,f], Armand Benssussan [c], Philippe Saiag [g], Maria Concetta Fargnoli [h], Ketty Peris [h], Jose A. Aviles [i], Ana Lluch [a], Gloria Ribas [a,*], Nadem Soufir [b,c,*]

[a] *Department of Haematology and Medical Oncology, Fundacion Investigacion Hospital Clinico-INCLIVA, Valencia, Spain*
[b] *Genetic and Biochemical laboratory, Bichat Claude Bernard Hospital, APHP, Paris 7 University, Paris, France*
[c] *Inserm U976, Cutaneous Research Center, Unit EA "Genetic Biomarkers of Skin Cancers", Saint Louis Hospital, Paris 7 University, Paris, France*
[d] *Institute of Biomedical Research, Alberto Sols, CSIC, University Autonoma of Madrid, Madrid, Spain*
[e] *Department of Dermatology, Bichat Claude Bernard Hospital, APHP, Paris, France*
[f] *Department of Dermatology, Saint Louis Hospital, APHP, Paris 7 University, Paris, France*
[g] *Department of Dermatology and Research Unit EA 4339 "Skin, environment, and cancer", Ambroise Paré University Hospital, Boulogne-Billancourt, APHP, University of Versailles-Saint Quentin en Yvelines, France*
[h] *Department of Dermatology, University of L'Aquila, L'Aquila, Italy*
[i] *Department of Dermatology, Gregorio Marañon Hospital, Madrid, Spain*

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Abstract ***Background and methods:*** Seven genetic biomarkers previously associated with melanoma were analysed in a meta-analysis conducted in three South European populations: five red hair colour (RHC) *MC1R* alleles, one *SLC45A2* variant (p.Phe374Leu) and one thermosensitive *TYR* variant (p.Arg402Gln). The study included 1639 melanoma patients and 1342 control subjects.

Results: The estimated odds ratio (OR) associated with carrying at least one *MC1R* RHC variant was 2.18 (95% confidence interval (CI): 1.86–2.55; p-value $= 1.02 \times 10^{-21}$), with an additive effect for carrying two RHC variants (OR: 5.02, 95% CI: 2.88–8.94, p-value $= 3.91 \times 10^{-8}$). The *SLC45A2* variant, p.Phe374Leu, was significantly and strongly protective for melanoma in the three South European populations studied, with an overall OR value of 0.41 (95% CI: 0.33–0.50; p-value $= 3.50 \times 10^{-17}$). The association with melanoma

* *Corresponding authors:* Address: Department of Haematology and Medical Oncology, Fundacion Investigacion Hospital Clinico, Av. Blasco Ibañez 17, 46010 Valencia, Spain. Tel.: +34 963862894; fax: +34 963987860 (G. Ribas), Laboratoire de Biochimie Hormonale et Génétique, Hôpital Bichat Claude Bernard, 46 rue Henri Huchard, 75018 Paris, France. Tel.: +33 140258551; fax: +33 140258785 (N. Soufir).
E-mail addresses: gribasdespuig@gmail.com (G. Ribas), nadem.soufir@bch.aphp.fr (N. Soufir).
[j] Both authors contributed equally.

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of the *TYR* variant p.Arg402Gln was also statistically significant (OR: 1.50; 95% CI: 1.11–2.04; p-value = 0.0089). Adjustment for all clinical potential confounders showed that melanoma risks attributable to *MC1R* and *SLC45A2* variants strongly persisted (OR: 2.01 95% CI: 1.49–2.72 and OR: 0.50, 95% CI: 0.31–0.80, respectively), while the association of *TYR* p.Arg402Gln was no longer significant. In addition, stratification of clinical melanoma risk factors showed that the risk of melanoma was strong in those individuals who did not have clinical risk factors.

Conclusion: In conclusion, our results show without ambiguity that in South European populations, *MC1R* RHC and *SCL45A2* p.Phe374Leu variants are strong melanoma risk predictors, notably in those individuals who would not be identified as high risk based on their phenotypes or exposures alone. The use of these biomarkers in clinical practice could be promising and warrants further discussion.

1. Introduction

Malignant melanoma (MM) incidence is currently increasing faster than that of any other malignancy in almost all Western countries.[1] This incidence is influenced by geographical parameters, with areas closer to the equator and higher in altitude generally having higher rates, indicating that ultraviolet radiation (UVR) plays an important role in the development of the disease.[1] Incidence is also higher in individuals with fair skin than in those with dark skin, suggesting that skin colour, which is known to be related to the degree of protection against UVR, is also important.[2,3] Therefore, genes involved in the determination of skin colour and tanning response are potentially implicated in MM predisposition, and may be useful predictors of MM risk in the general population.[4]

More than 120 genes involved in pigmentation processes, such as maturation, transport and distribution of melanosomes, have been identified through animal models.[5] However, only several genes have been identified as containing common genetic variants associated with human pigmentation in the normal range.[3,6–8] Recent genome-wide association studies (GWAS) have unveiled single nucleotide polymorphisms (SNPs) or genetic variants in *MC1R*, *TPCN2*, *ASIP*, *KITLG*, *NCKX5*, *TYR*, *IRF4*, *OCA2*, *SLC45A2* and *TYRP1* pigmentation genes. These findings emphasise the contribution of pigmentation pathways to melanoma predisposition and tumourigenesis through gene-environment interactions.[6–9]

The melanocortin-1 receptor gene (*MC1R,* [MIM# 155555]), located in chromosome 16q, encodes the melanocyte stimulating hormone receptor, a membrane bound protein central to pathways that signal the production of melanin. Inherited variation in *MC1R* is a robust genetic marker for increased risk of melanoma. The frequency of *MC1R* variants in the general population suggests that a considerable proportion of melanoma risk may be attributable to these genetic variants.[10]

Oculocutaneous albinism syndromes (OCA) consist essentially of a failure to synthesise melanin, the main contributor to human skin colour.[11,12] Mutations in each of the genes *OCA2* (MIM# 611409), *TYR* (MIM# 606933), *TYRP1* (MIM# 115501) and *SLC45A2* (also known as MATP (MIM# 606202)) (melanosome enzymes) are responsible for the four distinct OCA subtypes.[12]

The gene encoding tyrosinase (TYR, MIM# 606933) is located on chromosome 11q and consists of five exons coding for a 529 amino acids protein. Tyrosinase is one of the important enzymes that play a key role in pigmentation processes, catalysing the first two steps, and at least one subsequent step, in the conversion of tyrosine to melanin.[13] Mutations in this gene result in oculocutaneous albinism and skin pigmentation variation. One *TYR* polymorphism, p. Arg402Gln (rs1126809), has been described to be associated with eye colour and skin type.[14]

The *SLC45A2* gene is located on chromosome 5p, comprised of seven exons spanning 40 kb, and encodes a 530 amino acid protein presumably located in the melanosome membrane.[15] *SLC45A2* exhibits structural homology to plant sucrose-proton symporters and probably directs the traffic of melanosomal proteins and other substances to the melanosomes.[16] *SLC45A2* mutations cause pigmentation variation in the medaka,[17] mouse,[15,18] horse,[19] chicken and Japanese quail.[20] In humans, pathogenic mutations in *SLC45A2* lead to type IV oculocutaneous albinism. *SLC45A2* mutations disrupt tyrosinase processing and trafficking at the post-Golgi level.[21,22] Two human *SLC45A2* polymorphisms, p.Phe374Leu (rs16891982) and p.Glu272Lys (rs26722), 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 and recently, p.Phe 374Leu has been associated with melanoma.[11,23,24] Thus, the *SLC45A2* gene has been proposed as a melanoma susceptibility gene in light-skinned populations.[23,24]

As pigmentation characteristics and melanoma risk are closely correlated, we investigated the impact of these three pigmentation genes (*MC1R*, *TYR* and *SLC45A2*) on melanoma risk by performing a meta-analysis comprising three Mediterranean populations from

France, Italy and Spain. We point out the potential importance of pigmentation genes in MM susceptibility, and specifically, we confirmed the *SLC45A2* gene as a novel protective melanoma low penetrance susceptibility gene.

2. Patients and methods

2.1. Study subjects and data collection

An overall number of 1639 melanoma patients and 1342 control subjects were included in the study[23–26] comprising three different Mediterranean populations. One thousand thirty-seven French melanoma patients of which 238 familial melanoma patients and 117 multiple primary melanoma patients were recruited from the MELAN-COHORT including all melanoma patients from Departments of Dermatology of six different hospitals in Paris (Bichat, Percy, Ambroise Paré, Henri Mondor, Cochin and Saint-Louis hospitals). Two hundred and seventy-one Italian melanoma patients were enrolled at the Department of Dermatology of the University of L'Aquila, L'Aquila, Italy. Finally, 131 Spanish melanoma patients were recruited from the Department of Dermatology of Gregorio Marañón hospital in Madrid.

The control groups, matched by ethnicity and sex to the case group, were recruited among patients affected by diseases unrelated to melanoma attending the same hospitals and were composed of 925 French, 171 Italian and 245 Spanish control subjects with no personal or family history of skin cancer.

A standardised questionnaire was used to collect information on pigmentation characteristics such as eye, hair and skin colour, number of nevi, presence of solar lentigines, sun exposure habits and presence of childhood sunburns. Fitzpatrick's classification of skin type, tumour location, Breslow thickness and personal or family history of cancer was also included in the questionnaire. Fitzpatrick's classification of skin type was extracted from the medical record of cases only (Supplementary Table 1). The study was approved by the Local Ethical Committees and The Declaration of Helsinki Principles was followed. Informed consent was obtained from all the patients and control subjects enrolled in the study.

Genomic DNA from cases and controls was isolated from peripheral blood leukocytes using QIAamp Blood Mini Kit (QIAgen GmbH, Hilden, Germany) and MagNA Pure LC Instrument (according to the manufacturer's protocol [Roche Molecular Biochemicals AQ2, Mannheim, Germany]). Some samples were extracted using the traditional saline method.

2.2. Genotyping MC1R, SLC45A2 and TYR variants

MC1R, *SLC45A2* and *TYR* cDNA reference sequences with GeneBank accession numbers NM_002386.2, NM_016180.3 and NM_000372.4 were used.

The *MC1R* polymorphisms retained for genetic analysis were those associated with the red hair colour phenotype (RHC alleles), and included c.252 C>A p.Asp84Glu, c.425 G>A p.Arg142His, c.451 T>C p.Arg151Cys, c.478 C>T p.Arg160Trp and c.880 G>C p.Asp294His.[3,27,28] Details of the genes and SNPs studied, including MIM code, location, encoded protein, gene function, nucleotide changes and the context sequence are provided in Supplementary Table 2. French and Italian samples were genotyped for these SNPs using the KBiosciences PCR SNP genotyping system (KASPAR SNP Genotyping System KBiosciences, Hoddesdon Herts, United Kingdom (UK)), which is a homogeneous fluorescent genotyping system, using a unique form of allele-specific polymerase chain reaction (PCR) allowing allelic discrimination.[24,25] The *MC1R* coding region was amplified in the Spanish samples by polymerase chain reaction PCR using two overlapping pairs of primers which have been described previously[29] and the coding region was completely sequenced.[26]

We selected two non-synonymous SNPs described as being associated with melanoma risk in two low penetrance genes: c.1122 C>G p.Phe374Leu in *SLC45A2* and c.1707 G>A p.Arg402Gln in *TYR* (NCBI dbSNP rs16891982 and rs1126809, respectively)[23,24] to perform a meta-analysis. Details of the genes and SNPs studied, including MIM code, location, encoded protein, gene function, nucleotide changes and the context sequence are provided in Supplementary Table 2. For these two SNPs, French and Italian samples were genotyped by Kaspar technology[24] while Spanish samples were genotyped by Taqman technology[23] (Genotyping of the *TYR* variant, p.Arg402Gln, was not available for the Italian set). The PCR primers and probes were designated by Applied Biosystems (Foster City, CA) using their Custom Taqman SNP genotyping assays. All genotyping reactions were performed using TAQMAN SNP Genotyping Assay-allelic discrimination method (Applied Biosystems, Foster City, CA) (assay primers, probes and PCR conditions will be supplied upon request).

MC1R variants genotyping were successfully completed in 1397 patients and 1254 controls. SLC45A2 variant was genotyped efficiently in 1449 patients and 1327 controls. Finally, TYR variant was genotyped in 1172 patients and 1158 control subjects.

2.3. Statistical analysis

Statistical analyses were performed using the R software (version 2.12.2). Significance was declared at the 5% level. Hardy–Weinberg equilibrium has been checked for each genetic marker by applying of chi2 test for adjustment. Association was tested according to four modes of inheritance (additive, recessive, dominant and genotypic) with Logistic Regression (glm() R procedure) adjusted in both gender and age. All odds ratios (ORs) are reported with their 95% confidence interval (CI).

Considering the number of tests performed, and the high levels of associations, most of them would remain significant if a correction for multiple testing was applied. We conducted a meta-analysis in the French, the Italian and the Spanish populations by applying a Cochran–Mantel–Haenszel test,[30] as previously described.[31] Impact on pigmentation characteristics was further analysed by conducting analyses stratified on the various clinical melanoma risk factors. A Woolf test for homogeneity of the populations analysed was performed. Impact on pigmentation characteristics was further analysed by conducting multivariate analyses on the various clinical melanoma risk factors.

In order to specify the impact of each genetic variant on melanoma risk, we also calculate their population attributable fraction (PAF), defined as the proportion of disease cases in a population that is attributable to a particular exposure or cause. Using the relevant variables from the Poisson regression models, individual PAFs for each of the variables were calculated on the basis of RRs generated by new Poisson regression models with the variables classified dichotomously (exposed/unexposed). The population belonging to the lowest level of the variables in the initial Poisson regression models was defined as unexposed and the other levels were aggregated into the exposed group. The population-attributable fraction was calculated according to the formula $((RR - 1)/RR) \times$ the proportion of cases in the exposed population, where RR was the risk in the exposed population.[32] Interactions between risk factors were assessed by fitting a Logistic Regression with a multiplicative interaction term as implemented in Plink.[33]

3. Results

3.1. Association between SNPs genotypes and melanoma risk: meta-analysis

For this meta-analysis, we have used previously published results from the French and the Spanish data set.[23,24] Additional genotyping of *MC1R* and *SLC45A2* was performed in Italian patients and controls from l'Aquila, and genotyping of the *TYR* variant was conducted in all the French patients and controls. The meta-analysis comprised 1639 melanoma patients and 1343 cancer-free control subjects. Results are shown in Table 1.

The *MC1R* gene was only evaluated for the presence of RHC variants. These variants were significantly and strongly associated with melanoma risk, increasing seriously with the number of RHC alleles carried. The estimated OR associated with carrying at least one RHC variant was 2.18 (95% CI: 1.86–2.55, p-value $= 1.02 \times 10^{-21}$); however, OR for carrying two RHC variants was 5.02 (95% CI: 2.82–8.94, p-value $= 3.91 \times 10^{-8}$) (Data are shown in Fig. 1).

The p.Phe374Leu variant analysed in the *SLC45A2* gene was significantly and strongly protective for melanoma in the three South European populations studied, with an overall value of 0.41 (95% CI: 0.33–0.50, p-value $= 3.46 \times 10^{-17}$) (Results are shown in Fig. 2). Minor allele frequency (MAF) was 0.11 for France and Italian populations while MAF was 0.16 for the Spanish individuals.

Finally, association with melanoma and the *TYR* variant studied, p.Arg402Gln, was also statistically significant (OR: 1.50, 95% CI: 1.11–2.04, p-value = 0.0089) although the significance was not as high as for the previous two genes. (OR for each population studied are shown in Fig. 3).

3.2. Population attributable fraction (PAF)

The genetic risk factors considered for the calculation of the PAF were the presence of *MC1R* RHC variants, the presence of the C allele of the *SLC45A2* polymorphism (374Phe) and the presence of the A risk allele at the *TYR* variant (402Gln). We obtained a PAF of 21.4% for *MC1R* RHC variants, 29% for the *SLC45A2* variant and 1.89% for the *TYR* polymorphism.

3.3. Association between SNPs genotypes and phenotypic characteristics

We assessed whether *MC1R*, *SLC45A2* and *TYR* polymorphisms were associated with various phenotypic characteristics, after adjustment by age, sex and case–control status (Results are shown in Table 2). *MC1R* RHC variants were strongly associated with skin phototypes I–II, presence of ephelides, light hair colour and presence of solar lentigines, and moderately associated with light eye colour. The variant allele p.Phe374Leu of the *SLC45A2* gene was strongly associated with skin phototypes III–IV, and dark eye and hair colour, while it was moderately associated with the absence of ephelides and lentigines and with a low number of nevi. Finally, the variant allele p.Arg402Gln of the *TYR* gene was strongly associated with skin phototypes I–II, and moderately associated with light hair colour and the number of nevi.

3.4. Multivariate analyses

We considered hair and eye colour, skin phototype and the number of nevi as potential confounders in a multivariate model. Adjustment for all these potential confounders, plus age and sex, gave ORs shown in Supplementary Table 3. *SLC45A2* p.Phe374Leu polymorphism (OR = 0.50, 95% CI: 0.31–0.80, p-value = 0.004) and *MC1R* RHC variants (OR = 2.01, 95% CI: 1.49–2.72, p-value $= 5 \times 10^{-6}$) retained statistically

Table 1
Meta-analysis of genetic risk factors with Malignant Melanoma.

Gene	SNP	Genotypes	Cases *n* (%)	Controls *n* (%)	OR (95% CI)	*p*-Value[c]
A. Genotypic frequencies across MC1R, SLC45A2 and TYR genes and assessment of individual associations with Malignant Melanoma						
MC1R	RHC[a]	0/0	882 (63.1)	1011 (80.6)	2.18 (1.86–2.55)	**1.02×10^{-21}**
	variants[*]	1/0	440 (31.5)	229 (18.3)		
		1/1	75 (5.4)	14 (1.1)		
		MAF[b]	0.211	0.102		
SLC45A2	rs16891982[**]	CC	1329 (91.7)	1052 (79.3)	0.41 (0.33–0.50)	**3.50×10^{-17}**
	Phe374Leu	CG	110 (7.6)	243 (18.3)		
		GG	10 (0.7)	32 (2.4)		
		MAF[b]	0.045	0.116		
TYR	rs1126809[***]	GG	584 (49.8)	599 (51.7)	1.50 (1.11–2.04)	**0.0089**
	Arg402Gln	AG	475 (40.5)	482 (41.6)		
		AA	113 (9.7)	77 (6.7)		
		MAF[b]	0.299	0.275		
			pHWE	MAF Controls	MAF Cases	
B. Allele frequencies in cases and controls across the three Mediterranean populations						
MC1R	RHC[a]	France	0.68	0.11	0.22	**1.32×10^{-19}**
	variants[*]	Italy	0.57	0.10	0.20	**0.00068**
		Spain	0.22	0.07	0.13	**0.005**
SLC45A2	rs16891982[**]	France	0.0002	0.10	0.04	**5.07×10^{-17}**
	Phe374Leu	Italy	0.89	0.11	0.06	**0.011**
		Spain	0.60	0.16	0.07	**0.0006**
TYR	rs1126809[**]	France	0.07	0.28	0.30	**0.08**
	Arg402Gln	Spain	0.85	0.26	0.26	0.96

OR, odds ratio per minor allele; CI, confidence interval; SNP, single nucleotide polymorphisms.
Bold indicates statistically significant results.
[*] Sequencing was successfully performed in 1397 patients and 1254 controls.
[**] Genotyping was successfully performed in 1449 patients and 1327 controls.
[***] Genotyping was successfully performed in 1172 patients and 1158 controls.
[a] RHC variants are rs1805006 (c.252 C>A), rs11547464 (c.425 G>A), rs1805007 (c.451 C>G), rs1805008 (c.478 C>T) and rs1805009 (c.880 G>C).
[b] MAF, minor allele frequency.
[c] *p*-Value was calculated by Fisher's exact test.

significant results even when adjusted for all potential confounders.

3.5. Stratification on phenotypic risk factors

SLC45A2 and *MC1R* RHC variants were further analysed stratifying for phenotypic risk factors. The p.Phe374Leu *SLC45A2* change remained protective in fair phenotypic traits while *MC1R* RHC variants conferred risk even for those olive/dark complexion characteristics (Supplementary Table 4).

3.6. Interactions

We observed strong interaction with sunburns and eye colour with both *MC1R* ($p = 1.8 \times 10^{-3}$) and *SLC45A2* ($p = 0.036$) genes on melanoma risk. Furthermore, phototype and sunburns showed statistically significant interaction with the *SLC45A2* gene ($p = 7.5 \times 10^{-3}$) and hair colour with the *SLC45A2* and *TYR* genes ($p = 0.019$). Data is shown in Supplementary Table 5.

We further characterised the genetic interaction between risk *MC1R* RHC variants and protective *SLC45A2* allele. A dose effect reduction of *MC1R* RHC variants risk effect is observed when summing up the protective G allele of the *SLC45A2* p.Phe374Leu variant. Results are shown in Table 3.

4. Discussion

Human pigmentation pathways have been shown to play a crucial role in the pathogenesis of MM. Up to date, only the *MC1R* low penetrance gene was known to unequivocally account for a substantial variation in the incidence of MM. Nowadays, different studies have suggested that other pigmentation genes such as *SLC45A2*, *TYR*, *TYRP1*, *ASIP* and *OCA2* are also important in MM susceptibility.[23,24,34,35] Genetic variation across populations of different ethnic background, however, has yielded conflicting results concerning the role of these genes on melanoma susceptibility.

To clarify the role of three pigmentation genes, *MC1R*, *SLC45A2*, and *TYR* in melanoma susceptibility in a specific region, we conducted a meta-analysis concerning the role of several melanoma predisposing alleles (*MC1R* RHC alleles, *SLC45A2* p.Leu374Phe and *TYR* p.Arg402Gln) with melanoma risk in three

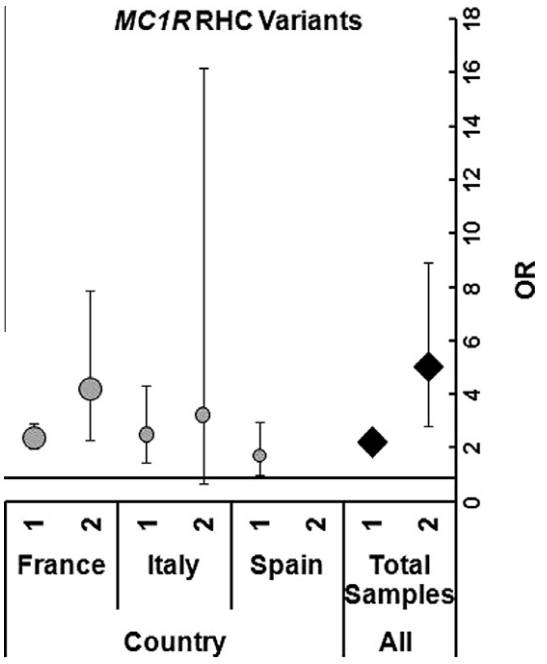

Fig. 1. Forest plot showing the association of carriers of one or two *MC1R* red hair colour (RHC) variants with melanoma risk. RHC variants are p.Asp84Glu, p.Arg142His, p.Arg151Cys, p.Arg160Trp, p.Asp294His. Dots represent odds ratio in the three South European populations. Diamond shapes represent pooled results for both stages. The size of the dot is proportional to the number of individuals, and error bars represent 95% confidence intervals. Eight Spanish melanoma cases have two RHC variants; however, the odds ratio is not available due to the absence of controls with two RHC variants.

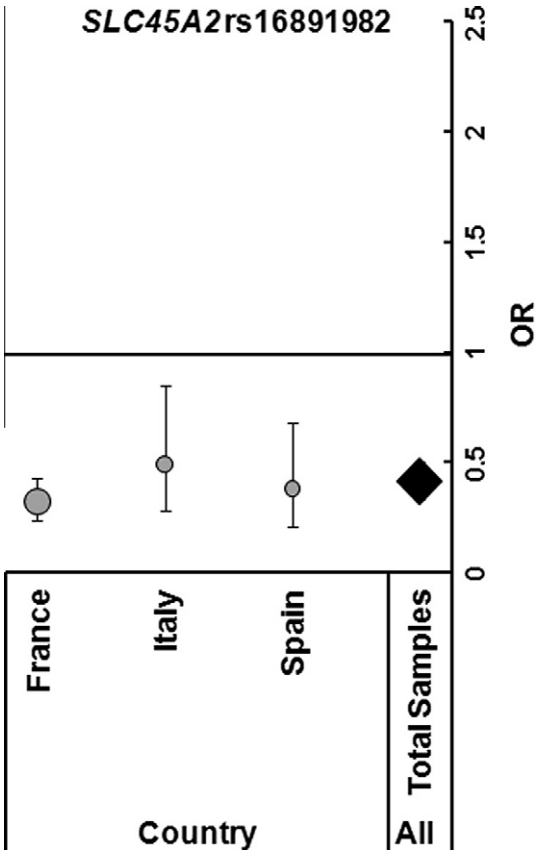

Fig. 2. Forest plot showing *SLC45A2* polymorphism, p.Phe374Leu, and melanoma protection. Dots represent odds ratio in the three South European populations. Diamond shapes represent the overall odds ratio. The size of the dot is proportional to the number of individuals, and error bars represent 95% confidence intervals.

South European regions (France, Italy and Spain). Meta-analysis represents a useful approach that leads to more conclusive results due to the pooled dataset, having greater power than each of the studies taken individually. Thus, 1639 melanoma cases and 1342 cancer-free control subjects were analysed. Furthermore, questions on whether the association of melanoma with genetic biomarkers may depend on the composition of the population under study, the country or the methodological features of the studies could be addressed by this meta-analysis.

MC1R was already known to have a role in melanoma susceptibility across Mediterranean populations[26,29,36–39] and its implication is strongly confirmed in the current study. The PAF of melanoma associated with *MC1R* RHC variants has been shown to be important in Mediterranean Europe (16%) compared to Northern Europe (9%),[37] which is also confirmed in this meta-analysis, where the PAF was calculated to be 21%, close to the one calculated by Williams et al. (16%).[37] In addition, our results clearly indicate that *MC1R* RHC alleles are strong melanoma risk factors that appear to be independent of the presence of clinical melanoma risk factors. This is in concordance with previous studies from France,[29] Greece,[36] Italy[25] and Spain.[26,38] Melanoma risk attributable to *MC1R* may arise through the determination of the tanning response of skin to UV light, which can then either ameliorate or exacerbate the genotoxic effects of sunlight. Nevertheless, the relationship between some *MC1R* variants and melanoma in darkly-pigmented Caucasian populations suggests the MC1R signalling pathway may have an additional role in skin carcinogenesis beyond the UV-filtering differences between dark and fair skin.

We observed a strong association of *MC1R* with MM, even after stratification on fair pigmentation characteristics (i.e. pale skin and skin type I-II, see Supplementary Table 4). By opposite, in the Australian population, Palmer et al. observed an association of *MC1R* variants with melanoma only in darkly pigmented people,[40] suggesting different role or mechanisms for *MC1R* variants in these populations regarding melanoma risk. Recently, Kanetsky et al. suggested that *MC1R* genotyping provides information about melanoma risk in those individuals that would not be identified as high risk based on their phenotype.[41] In our study, RHC alleles were associated with melanoma risk after stratification either for the presence of melanoma clinical risk factors (i.e. fair pigmentation characteristics), or in their absence (i.e. dark skin pigmentation, skin type 3–4, dark hair and eye colour

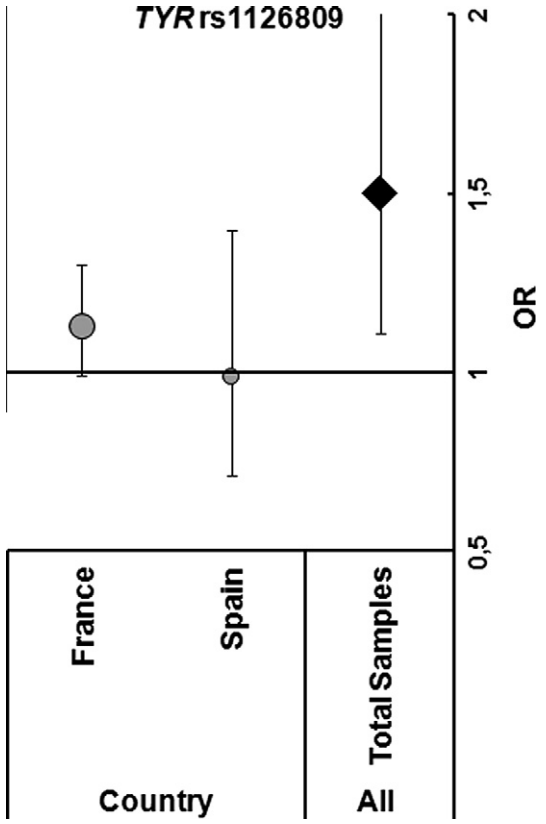

Fig. 3. Forest plot showing *TYR* polymorphism, p.Arg402Gln, and melanoma risk predisposition. Dots represent odds ratio in the three South European populations. Diamond shapes represent the overall odds ratio. The size of the dot is proportional to the number of individuals, and error bars represent 95% confidence intervals.

and absence of ephelides). Consequently, given the relatively high PAF (21%) and their independent effect on MM risk, discussions should be opened on whether *MC1R* RHC variants could be used as melanoma predictive biomarkers in populations from the south of Europe.

In 2008, a novel genetic biomarker in the *SLC45A2* gene, rs16891982, was identified as being associated with a genetic predisposition to melanoma.[23,24] This variant encodes a non-synonymous amino acid change and has previously been reported to be strongly associated with ethnic ancestry and normal human pigmentation variation.[11] In this meta-analysis, we state the role of this variant for melanoma susceptibility, and as shown for *MC1R*, the effect persists after the adjustment for pigmentation characteristics, protecting even those individuals with a fair phenotype. Leu374 variant confers a protective effect against melanoma in Caucasian populations. The PAF to the p.Leu374Phe variant was particularly high (28%), emphasising the role of this variant in the multifactorial susceptibility to melanoma. There was a decreasing gradient of Leu374 allele frequencies from the Northern Africa to Europe. In our study, the Leu374 allelic MAF was 0.16 in Spanish controls while a slight decrease is observed in French and Italian controls (0.11). The Leu374 MAF in cases was lower, ranging from 0.04 in French melanoma patients to 0.07 in Spanish ones. However, the role of the variant Leu374 in the predisposition to melanoma seems to vary across different countries. For example, this variant was not associated to melanoma in Iceland,[42] where the MAF is 0.02 in the general population. However, it has been consistently associated with MM in other countries such as the Netherlands, Sweden, Austria and Australia.[42,43]

Finally, the *TYR* p.Arg402Gln variant has previously been associated with melanoma in GWAS studies performed in Caucasian populations.[9] Its association with melanoma is confirmed in the current meta-analysis, although the Gln402 variant seems not to be as strongly associated with melanoma as the other two genes studied. The lower impact of this variant on melanoma susceptibility was confirmed by its low PAF (<2%) compared to those for *SLC45A2* and *MC1R*.

In conclusion, our results show without ambiguity that in South Europe, *MC1R* RHC and *SCL45A2* p.Phe374Leu variants are strong melanoma risk predictors independent of clinical characteristics. Whether or not these biomarkers could be used in clinical practice warrants further discussion.

Table 2
Association between single nucleotide polymorphisms (SNPs) and various phenotypic characteristics after adjustment on age, sex and case control status.

Characteristic	*MC1R* RHC variants*		*SLC45A2* rs16891982		*TYR* rs1126809	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Eye colour	1.48 (1.25–1.75)	**5.0×10^{-6}**	0.36 (0.29–0.46)	**4.6×10^{-17}**	1.09 (0.78–1.53)	0.60
Hair colour	2.24 (1.89–2.64)	**2.7×10^{-21}**	0.34 (0.27–0.42)	**1.5×10^{-21}**	1.65 (1.20–2.26)	**2.1×10^{-3}**
Number of Nevi	1.14 (0.95–1.36)	0.16	0.56 (0.42–0.75)	**8.2×10^{-5}**	1.42 (1.01–2.00)	**0.04**
Presence/Absence of lentigines	1.70 (1.38–2.10)	**9.6×10^{-7}**	0.73 (0.55–0.96)	**0.03**	0.96 (0.64–1.43)	0.84
Presence/Absence of ephelides	3.94 (3.23–4.80)	**5.41×10^{-42}**	0.44 (0.31–0.61)	**1.06×10^{-6}**	1.18 (0.82–1.69)	0.37
Fitzpatrick's phototype	2.80 (2.36–3.33)	**8.6×10^{-32}**	0.28 (0.21–0.36)	**2.2×10^{-20}**	2.11 (1.51–2.94)	**1.0×10^{-5}**

OR, odds ratio per minor allele; CI, confidence interval.
Bold indicates statistically significant results.
* RHC variants are rs1805006 (c.252 C>A), rs11547464 (c.425 G>A), rs1805007 (c.451 C>G), rs1805008 (c.478 C>T) and rs1805009 (c.880 G>C).

Table 3
Interactions between protective (*SLC45A2*) and risk (*MC1R*) variants and their effect on Melanoma susceptibility.

Joint genotype		Cases	Controls	*N*	OR (95% CI)	*p*-Value
MC1R RHC* variants	*SLC45A2* rs16891982					
0/0	CC	740	794	1534	1.00	REF
	G/–	70	206	276	0.36 (0.27–0.49)	**$<1 \times 10^{-4}$**
0/1	CC	364	185	549	2.11 (1.72–2.59)	**$<1 \times 10^{-4}$**
	G/–	34	42	76	0.87 (0.55–1.38)	0.56
1/1	CC	59	11	70	5.75 (3.03–10.92)	**$<1 \times 10^{-4}$**
	G/–	3	3	6	1.07 (0.25–4.66)	1.00

OR, odds ratio per minor allele; CI, confidence interval; REF, reference value.
Bold indicates statistically significant results.
* RHC variants are rs1805006 (c.252 C>A), rs11547464 (c.425 G>A), rs1805007 (c.451 C>G), rs1805008 (c.478 C>T) and rs1805009 (c.880 G>C).

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2012.03.006.

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