

## MELANOMA, ANCESTRY AND MC1R VARIANTS IN THE URUGUAYAN ADMIXED POPULATION

## MELANOMA, ANCESTRALIDAD Y VARIANTES MC1R EN LA POBLACIÓN MESTIZADA URUGUAYA

Hochmann J.<sup>1</sup>, Cappetta M.<sup>1</sup>, Pérez J.<sup>2</sup>, Colistro V.<sup>1</sup>, Nicolleti S.<sup>2</sup>, Larre Borges A.<sup>2</sup>, Ribas G.<sup>3</sup>, Martínez Asuaga M.<sup>2</sup>, Bertoni B.<sup>1</sup>

<sup>1</sup>Departamento de Genética, Facultad de Medicina, Universidad de la República, Montevideo-Uruguay.

<sup>2</sup>Cátedra de Dermatología, Hospital de Clínicas, Universidad de la República, Montevideo-Uruguay.

<sup>3</sup>Fundación para la Investigación del Hospital Clínico de Valencia-INCLIVA, Valencia-España.

\*Corresponding author: bbertoni@fmed.edu.uy

### ABSTRACT

Malignant melanoma (MM) is the most dangerous type of skin cancer and the main cause of death produced by skin diseases. In Uruguay, the incidence rate is 3.8/100,000, one of the highest in Latin America. We analyzed the contribution of ancestry and MC1R as a candidate gene for sporadic melanoma in Uruguay. Our objective was to investigate the possible associations between ancestry and the MC1R gene with sporadic melanoma in the Uruguayan population. To that end, one hundred patients with sporadic MM and 107 controls were recruited. Phenotypic factors and lifestyle were evaluated as risk factors. At the same time, we analyzed five ancestry informative markers, the MC1R variants (R151, R160 and D294H) and five tag-SNPs. Phototype, atypical nevi, sunburns and recreational exposure were the main risk factors for MM in the Uruguayan population. We confirmed 16q as a candidate region for MM. R151C, and R160W showed an important association with risk of melanoma (OR= 3.85, P=  $1 \times 10^{-2}$ ; OR= 10.15, P=  $7 \times 10^{-3}$ , respectively). Furthermore, three novel MC1R haplotypes from the promoter region were detected, and the two most common haplotypes for the coding region were different to the ones found in Europeans through HapMap. However, MC1R coding region haplotypes revealed a highly similar frequency to that of the Spanish population. Our results showed that the chromosomal 16q region confers susceptibility to MM risk in the Uruguayan population. In addition, the admixed genome structure of the MC1R region could be part of the explanation of melanoma etiology.

**Key words:** melanoma susceptibility, Melanocortin 1 Receptor gene, Red Hair Color (RHC) mutations, ancestry, Uruguayan population.

### RESUMEN

El melanoma maligno (MM) es uno de los más peligrosos, y la principal causa de muerte producida por tumores de piel. En el Uruguay, la tasa de incidencia es de 3,8/100.000, una de las más altas de América Latina. En este trabajo analizamos la ancestría y el gen candidato MC1R entre los pacientes con MM del Uruguay. Nuestro objetivo fue investigar la posible asociación entre ancestría y el gen MC1R en pacientes con melanoma esporádico en la población uruguaya. Con tal finalidad, se reclutaron 100 pacientes con MM esporádico y 107 controles. Se evaluó el riesgo de factores fenotípicos y de estilo de vida. Además se analizaron cinco marcadores informativos de ancestría, variantes del gen MC1R (R151, R160 y D294H) y cinco tagSNPs. El fototipo, los nevos atípicos, quemaduras solares y la exposición recreativa fueron los principales factores de riesgo para MM en la población uruguaya. La región cromosómica 16q es candidata para MM, mientras que R151C y R160W mostraron una importante asociación con el riesgo para MM (OR= 3,85, P=  $1 \times 10^{-2}$ ; OR= 10,15, P=  $7 \times 10^{-3}$ , respectivamente). Por otra parte, se detectaron tres nuevos haplotipos en la región promotora y los dos haplotipos más frecuentes en la región codificante son diferentes a los encontrados en la población europea. Sin embargo, los haplotipos de la región codificante presentan una frecuencia muy similar a las encontradas en la población española. Los resultados muestran que la región cromosómica 16q confiere susceptibilidad al riesgo de MM en la población uruguaya. Por otra parte, la estructura genómica mestizada de la región del MC1R podría explicar la etiología del melanoma.

**Palabras clave:** susceptibilidad a melanoma, gene Receptor de Melanocortina 1, mutaciones Red Hair Color (RHC), ancestralidad, población uruguaya.

Fecha de recepción: 15/05/2015

Fecha de aceptación de versión final: 29/02/2016

## INTRODUCTION

Melanoma is the most dangerous type of skin cancer and the main cause of death produced by skin diseases. It develops from melanocytes, but can also begin in other pigmented tissues, such as the lymph nodes, eye or the intestines. The overall incidence of malignant melanoma (MM) has been increasing continuously for the last four decades in European and European descendant populations (Garbe and Leiter, 2009). In Uruguay, the incidence rates adjusted by age is 4.5 and 3.5 per 100,000 in men and women respectively, and it is clearly on the rise since the previous study conducted in 1996 (Barrios *et al.*, 2001).

The etiology of MM remains unclear but it is known that both genetic and environmental factors influence the development of this sporadic disease (Bataille, 2003; Scherer and Kumar, 2010). Familial predisposition to melanoma, which is considered in families with two or more first-degree relatives with MM, is believed to be responsible for approximately 10% of all MM cases (Larre Borges *et al.*, 2009). High-risk alleles are often expressed as familial clusters, whereas lower-risk alleles result in sporadic cases. The CDKN2A and CDK4 are susceptibility genes with high penetrance, which account for approximately 20% to 57% of disease susceptibility in family melanoma (Bloethner *et al.*, 2009; Goldstein *et al.*, 2007).

Melanocortin-1 Receptor (MC1R) is considered to be a susceptibility gene with low penetrance. This gene is implicated in human pigmentation and encodes a G-protein-coupled receptor which is expressed in melanocytes. MC1R is located in chromosome region 16q24, with an open reading frame of 951 bp and, until recently, was thought to be composed of a single exon (MIM #155555).

The MC1R gene is highly polymorphic, with most allelic variation being restricted to European populations, followed by Asian and, finally, African populations (Gerstenblith *et al.*, 2007; Savage *et al.*, 2008). A group of missense mutations have been associated with red hair, fair skin, freckles and poor tanning ability, and these variants are referred to as “red hair colour” (RHC) variants (Duffy *et al.*, 2004; Wong and Rees, 2005). In North European populations, the association between MM, red hair color, fair skin and RHC variants is significant (Bastiaens *et al.*, 2001; Kanetsky *et al.*, 2004; Palmer *et al.*, 2000). However, among Southern European populations the association

between RHC variants and melanoma is not clear. In Mediterranean populations, RHC variants are not clearly associated either with skin pigmentation or hair colour, but they show an association with MM (Fernandez *et al.*, 2007; Landi *et al.*, 2005; Stratigos *et al.*, 2006). Melanoma research focused on European or European descendant populations could represent a pitfall to discriminate gene-gene interactions as previously described. In this sense, admixed populations are becoming a source to unveil genes associated to complex disease (Bonilla *et al.*, 2004a; Darvasi and Shifman, 2005). The mixed genome structure could help to discriminate the gene effects in complex diseases such as cancer. However, there is little information on melanoma in Latin America. With respect to sporadic MM, the incidence rate ranges from 1.8 in Peru to 6.5 in Sao Paulo, Brazil, with an average incidence rate estimated in 4.5 for Latin American populations (Erdei and Torres, 2010; Schmerling *et al.*, 2011; Sortino-Rachou and Curado, 2011). These incidence rates are lower than those found in European populations but higher than the ones found in African populations (one of the parental contributors to Latin American admixed populations). However, few studies have been carried out in Latin America to try to get an understanding on the genes involved in sporadic MM. In Uruguay, the presence of germline mutations in the CDKN2A and MC1R genes was reported in families with hereditary melanoma (Larre Borges *et al.*, 2009). The authors also detected RHC variants in these families and a novel CDKN2A mutation was described, which was probably due to a founder effect. These results reinforce the idea that sporadic melanoma should be further investigated.

Our study is the first to analyze risk factors, contribution of parental populations, RHC variants and haplotypes of the MC1R gene and their association with sporadic MM in an admixed Uruguayan population.

## MATERIALS AND METHODS

### *Study population and data collection*

A total of 100 consecutive and non-related sporadic MM cases were recruited in a four year period from May 2006 to May 2010 from the Dermatology Department at Clinical Hospital “Manuel Quintela” (Montevideo). The diagnosis of all patients had been confirmed by histopathological

analysis. All patients had not first-grade relatives affected by skin cancers, and underwent skin examination by a trained dermatologist to record the number and type of nevi and to define tumor location. During the same period, a control sample was established with 107 individuals matched by sex- and age, without personal or family history of skin cancer, from the Clinical analysis Laboratory of the same medical institution.

Following informed consent of all participants in the study, blood samples were obtained for DNA extraction, and participants answered an interview-based questionnaire and underwent physical examination to record information about pigmentation characteristics (eye, hair and skin colour, presence of solar lentigines), Fitzpatrick's classification of skin type, personal and family history of cancer, and environmental risk factors such as history of sunburns, sun exposure, dietary habits, chemical exposure, among others.

For statistical analysis we measured the following phenotypic variables: number of atypical, congenital and acquired nevi, and presence of lentigines. Regarding the environmental variables, we considered four categories of sun exposure: i) on vacation until 15 years old; ii) during the rest of the year at 15 years old; iii) on vacation during adulthood; and iv) during the rest of the year during adulthood. Regarding sunburns, we considered four periods of life: before age 10, between ages 10 and 15, between ages 16 and 20, and after age 20.

The protocol of this study and the informed consent were approved by the Ethics Committee of the Medicine School of Universidad de la República; Montevideo, Uruguay.

#### Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using FlexiGene® DNA Kit (QIAGEN) and stored at  $-20^{\circ}\text{C}$  until analysis.

PV92 (rs3138523) and APOA1 (rs3138522) were genotyped by PCR amplification and conventional agarose gel electrophoresis as described in Bonilla *et al.* (2004b). TSC111025 (rs2065160), SLC24A5 (rs1426654), GC1F (rs4588), and the four genetic variants of MC1R related to the RHC phenotype (R151C, R160W and D294H) were assayed using ABI PRISM® SNaPshot™ Multiplex System (Applied Biosystem). The amplicon primers and the oligonucleotides used for the extension reactions are

described in Supplementary Table 1. The m-fold server was used to assess the secondary structure of the PCR products and the accessibility of the SNaPshot primers (<http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form>).

Five SNPs in the MC1R region selected by Ibarrola-Villava *et al.* (2010), were chosen from the HapMap International Project database by using HaploView v4.2 software (Barrett *et al.*, 2005), and taking into account tagSNPs from the European, African (Yoruba) and Asian (Han Chinese and Japanese) subset of data. According to Ibarrola-Villava's *et al.*, (2010) work, two haploblocks were defined to make interpopulation comparisons (). Block 1 included the SNPs rs8045560, rs2270459 and rs3212346, and was located within the putative promoter region, whereas Block 2 included rs3212363 and rs3212369 and extended along the transcript region. These HapMap SNPs were genotyped by KASPar SNP Genotyping System (Kbiosciences, UK).

#### Statistical methods

Allele frequencies were calculated by allele count, and the Hardy Weinberg equilibrium was tested for each SNP by using SNPSTAT's software (Solé *et al.*, 2006). The risk of MM associated with SNPs and AIMs or other determinants was estimated by fitting conditional logistic regression models, and was expressed as the odds ratio (OR) and 95% confidence interval (CI). All p-values cited are two-sided and  $p < 0.05$  is regarded as statistically significant after the Bonferroni correction. We used the PLINK (Purcell *et al.*, 2007) and Epi Info v3.5 (Centers for Disease Control and Prevention, Atlanta, USA) programs in order to calculate the association between the variables. We also used the Haploview v4.3 program to study the association between MC1R haplotypes and their relation to the disease.

Admixture was estimated by the Gene Identity method (Chakraborty, 1985) implemented in ADMIX95 program designed by B. Bertoni (available at <http://www.genetica.fmed.edu.uy/software.htm>).

## RESULTS

Two hundred and seven individuals were selected from the Uruguayan population for this study. Cases were represented by 100 patients with sporadic melanoma, and

**Table 1.** Demographic characteristics, phototypes, sun exposure pattern and type of nevi of the studied sample.

Phenotypic characteristics	Cases	Controls	p-value	OR (95% CI)
<b>Sex (%)</b>				
Female	60 (60.0)	64 (59.8)	n.s.	1.0 (0.6-1.7)
Male	40 (40.0)	43 (39.2)		
<b>Age (SD)</b>	56.6 (15.0)	62.4 (12.5)	< 0.01	
<b>Phototype I/II (%)</b>	64 (64.0)	17 (15.8)	< 0.001	5.8 (2.9-11.8)
<b>Congenital nevi (%)</b>	20 (20.0)	10 (9.3)	< 0.05	2.5 (1.1-5.6)
<b>Atypical nevi (%)</b>	48 (48.0)	4 (3.7)	< 0.001	10.1 (4.1-24.9)
<b>History of atypical nevi (%)</b>	41 (41.0)	12 (11.2)	< 0.001	4.6 (2.0-10.5)
<b>Acquired nevi (%)</b>			< 0.001	
<5mm	84 (84.0)	80 (73.0)		
>5mm	9 (9.0)	1 (0.9)		
0	7 (7.0)	28 (25.7)		
<b>Acquired nevi (SD)*</b>	30.6 (26.7)	16.6 (15.3)	< 0.001	
<b>Sun exposure (%)</b>				
<15 years old	42 (42.0)	39 (35.7)	n.s.	1.4 (0.7-2.6)
Adulthood	35 (35.0)	33 (30.2)	n.s.	1.1 (0.6-2.1)
<b>Recreational exposure (%)</b>				
< 15 years old Recreational Exp.	67 (67.0)	45 (41.2)	< 0.001	2.8 (1.4-5.3)
Adulthood Recreational Exp.	61 (61.0)	37 (33.9)	< 0.05	2.1 (1.1-3.9)
<b>Sunburns (%)</b>				
Before 10 years old	26 (26.0)	6 (5.5)	< 0.001	4.9 (1.8-13.2)
Between 10-15 years old	36 (36.0)	11 (10.3)	< 0.001	4.1 (1.9-9.2)
Between 15-20 years old	38 (38.0)	23 (21.4)	< 0.05	1.9 (1.0-3.8)
Greater 20 years old	32 (32.0)	18 (16.8)	< 0.05	2.0 (1.0-4.2)
<b>Atypical nevi (%)</b>			< 0.001	
0	53 (53.0)	100 (93.5)		
11-10	36 (36.0)	7 (6.5)		
11-25	9 (9.0)	-		
26-40	2 (2.0)	-		

one hundred and seven individuals without melanoma acted as controls. The average age of the patients was 57 years, slightly younger than the control sample (62 years). Since more female cases were detected during the sampling period, the control sample was matched in this proportion (Table 1).

#### Risk factors

As it can be seen in Table 1, vacation sun exposure in childhood (OR= 2.76, P= 0.001), phototype I/II (OR=

5.84, P=  $1 \times 10^{-9}$ ), presence of atypical nevi (OR= 10.11, P=  $1 \times 10^{-9}$ ), congenital nevi (OR= 2.88, P= 0.02), acquired nevi (P=  $1.3 \times 10^{-8}$ ), family history of atypical nevi (OR= 4.6, P=  $1 \times 10^{-4}$ ), and sunburn at 15 years old (OR= 4.13, P= 0.0008) were the main risk factors for sporadic MM in the Uruguayan population sample.

#### Ancestry and AIMs analysis

The grandparent's geographical origin could be recorded in 90 cases and 102 controls. In Table 2, it can be seen

**Table 2.** Geographical origin of the grandparents of cases and controls.

Geographic origin	Cases (%) N=360	Controls (%) N=408
Europe	36.6	17.2
Latin America <sup>(a)</sup>	46.1	63.7
Unknown	17.3	19.1

**Table 3.** Analysis of admixture in the Uruguayan population. Percentage of the parental population contribution to the population samples.

Origin	Parental population contribution	
	Cases	Controls
European	82.47	76.51
Native American	0.00	3.42
African	17.53	20.07
R2*	0.97	0.99

that European ancestry was more frequent in cases than in the control population, 36.6% and 17.2% respectively ( $P < 0.05$ ). Latin American grandparents were born in Uruguay or other Latin American countries and declared not to belong to any Native American ethnic group.

Five Ancestry Informative Markers (AIMs) were selected and analyzed in order to determine the degree of admixture of the studied sample (Table 3). The selected AIMs exhibited a high level of allele frequency difference among parental populations (European, West African and Native American). All SNP genotypes were in Hardy-Weinberg equilibrium with the exception of GC1F. In the admixture analysis, the European contribution was 82% in MM patients and 77% in controls. No Native American contribution was detected in MM patients and only a 3% contribution was found in controls (Table 3). The individual analysis of AIMs indicated a significant association between PV92 and TSC111025 with MM risk ( $P = 0.006$  and  $P = 0.027$ , respectively). However, for others markers we did not detect any significant association with the disease (Table 4).

#### *RHC and haplotypes variants*

Considering the fact that PV92 (16q23.3) is relatively close to MC1R gene (16q24.3), we decided to study this

chromosomal region that overlaps with the MC1R gene. We analyzed three strong RHC variants: R151C, R160W and D294H. Among the patients, homozygous individuals for the R160W and R151C mutants were detected. However, we only detected heterozygous individuals for the D294H mutation. Only the two gene variants R151C and R160W were associated with melanoma risk ( $OR = 3.85$ ,  $P = 0.01$  and  $OR = 10.15$ ,  $P = 0.007$ , respectively). The RHC variants (R151C, R160W, and D294H) were present at frequencies of 2%, 0.5%, and 1% respectively in the control sample (Table 4).

There was only one red haired individual among the control sample, but 10 (9.5%) of MM cases had red hair. Among these ten red-haired cases, eight carried at least one functional MC1R variant.

As for the five tag-SNPs of MC1R studied, no association with MM risk was detected (Table 4). Two haploblocks were constructed in order to compare the results of this study with those obtained in a previous work involving a Spanish population (Ibarrola-Villava *et al.*, 2010). Block 1 corresponds to the SNPs located at the promoter region and Block 2 corresponds to the transcription region of the MC1R gene. The analysis showed no association with MM risk. However, haplotype frequencies in the Uruguayan population were clearly different from those found in the Spanish population (Table 5). In the Uruguayan population we detected seven haplotypes. Three of them are exclusive of our population (TGA, CTG, TTG) because they were not described in any of the studied HapMap populations. The haplotype CGA, which is present in the European and Yoruba populations, was detected at lower frequency. Furthermore, we detected another two haplotypes (CGG, TGG) in the Uruguayan population whose frequencies differ from those in European and Spanish populations. Finally, the remaining haplotype (CTA) was detected only in the controls, with similar frequency to the one found in the European population. On the other hand, the frequencies of the Uruguayan block 2 haplotypes were similar to those of the Spanish population.

Finally, the haplotype (CGCCGGAA) which included the R151C, R160W and D294H loci and the five tag-SNPs previously mentioned was present in frequencies of 18% and 4% among controls and cases respectively (data not shown). This haplotype is a combination of the wild type RHC variants and the most common haplotypes from Block 1 (CGG) and Block 2 (AA), and is protective for the disease ( $OR = 0.21$ , 95% IC (0.057-0.81),  $P = 0.026$ ).

**Table 4.** Association between AIMs, RHC variants, SNPs of the MC1R gene and MM risk.

SNP	Allele	Chromosome location	N	Cases MAF	Control MAF	HW E	OR (95 % CI)	p-value
AIMs								
GC1F	G	4q13.3	214	0.420	0.500	0.008	1.32 (0.49- 3.57)	0.220
SLC24A5	G	15q21.1	228	0.270	0.130	1.000	1.22 (0.47- 3.22)	0.004
TSC111025	C	1q32.1	214	0.190	0.320	0.130	2.97 (1.31- 6.70)	0.027
PV92	+	16q23.3	342	0.080	0.180	1.000	3.63 (1.55- 8.53)	0.006
APOA1	-	11q23.3	344	0.040	0.040	1.000	1.01 (0.33- 3.04)	0.990
RHC variants								
R151C	T	16q24.3	414	0.070	0.020	1.000	3.85 (1.25-11.8)	0.011
D294H	C	16q24.3	412	0.030	0.010	1.000	1.95 (0.48-7.91)	0.340
R160W	T	16q24.3	414	0.050	0.005	1.000	10.15 (1.28-80.0)	0.007
MC1R's SNPs								
rs8045560	T	16q24.3	322	0.460	0.480	0.390	0.94 (0.44-1.98)	0.750
rs2270459	T	16q24.3	336	0.040	0.040	1.000	1.37 (0.46-4.03)	0.570
rs3212346	A	16q24.3	336	0.060	0.060	0.010	1.07 (0.42-2.73)	0.890
rs3212363	T	16q24.3	338	0.300	0.300	0.800	1.23 (0.64-2.35)	0.730
rs3212369	G	16q24.3	324	0.180	0.180	0.730	1.32 (0.66-2.65)	0.450

**Table 5.** Haplotype frequencies of MC1R's SNPs in the HapMap populations (Europe, Africa and Asia), Spain, and Uruguay.

MC1R haplotypes	Populations							p-value
	HapMap			Spain		Uruguay		
	Europeans	Africans	Asians	Cases	Controls	Cases	Controls	
<b>Block 1</b>								
CGG	36.7	54.2	65.0	32.2	33.2	52.1	58.1	1.00
TGG	55.8	3.3	10.5	59.0	59.4	38.2	33.9	0.12
CTA	5.8	21.7	21.8	5.7	6.5	....	3.6	0.34
CGA	1.7	20.9	....	3.1	0.5	1.7	1.6	0.87
TGA	....	....	....	....	....	4.8	1.6	0.17
CTG	....	....	....	....	....	....	1.3	0.21
TTG	....	....	....	....	....	2.2	....	....
<b>Block 2</b>								
AA	54.8	3.5	13.1	58.1	56.2	51.2	54.3	1.00
TA	31.3	57.3	65.5	21.6	23.7	31.7	28.7	0.58
AG	13.7	39.2	21.4	20.3	19.9	17.1	17.0	0.87

## DISCUSSION

Although numerous studies about the genetic risk factors implicated in sporadic melanoma have been carried out in different populations (Raimondi *et al.*, 2008), most of them were conducted on European populations or European population descendants. As far as we know, our study is the first one to analyze risk factors and also RHC variants and haplotype structure of the MC1R gene in a

hybrid population of Latin America and their associations with sporadic melanoma.

The presence of phototype I and II, solar exposition in childhood during vacations and the atypical nevi are the main phenotypic MM risk factors in the Uruguayan population. These results are consistent with previous studies involving populations of European ancestry, but

also with those obtained in the admixed populations of Argentina and Southern Brazil (Bakos *et al.*, 2009; Lascano *et al.*, 2004; Luiz *et al.*, 2012). The solar sunburns suffered until 15 years of age showed an important association with MM risk. However, it is not an important risk factor in Southern Brazil (Bakos *et al.*, 2002), Argentina (Lascano *et al.*, 2004), or even in Southern Europe (Nikolaou *et al.*, 2008), which suggest a different and more risky behavior among the Uruguayan population.

The high incidence of sporadic MM among Europeans or European descendants is well documented as it was previously stated. In the Uruguayan MM patients, an excess of European ancestry was detected by two different approaches. From a demographic point of view, the number of grandparents of European origin among the patients was higher than in controls. Similarly, in two Latin American studies, one performed in Argentina and the other in Brazil, the presence of European grandparents was detected as a risk factor for MM (Bakos *et al.*, 2009; Lascano *et al.*, 2004). A similar approach was developed in a study involving Alabama melanoma patients showing a predominant Celtic ancestry of grandparents (Acton *et al.*, 2004). This observation suggests that an important part of the patients' genome is of European origin. However, in Latin America, personal information is not sufficient to assign a person or group to an ancestral origin. Definition by skin color or ancestry represents a very heterogeneous group of people from the genetic point of view as it was demonstrated in Brazil (Pimenta *et al.*, 2006; Suarez-Kurtz *et al.*, 2007). In our study, we analyzed genetic ancestral contribution and found a slight difference between European ancestry cases were compared with controls. Furthermore, no Native American contribution was detected in the patients. Previous studies found a trihybrid structure of the Uruguayan population with European, Native American and African contributions (Hidalgo *et al.*, 2005; Sans *et al.*, 1997).

The individual analysis of the AIMs showed a significant association of TSC111025 and PV92 with sporadic melanoma. The former, in the 1q32.1, was described previously in Puerto Rican women when ancestry and skin pigmentation were analyzed (Bonilla *et al.*, 2004b). This region includes gene such as MDM4, a p53 binding protein homolog, (Gene ID: 4194), leucine rich repeat neuronal 2 (LRRN2–Gene ID: 10446) or phosphoinositide-3-kinase, class 2, beta polypeptide (PIK3C2B–Gene ID: 5287), all of them related to oncogenic transformation.

On the other hand, PV92 is located in chromosome region 16q23.3 close to the MC1R gene (16q24.3), a candidate gene with a minor effect in the etiology of sporadic melanoma (Kanetsky *et al.*, 2002; Landi *et al.*, 2005; Palmer *et al.*, 2000). The R151C, R160W, and D294H variants have been described previously as related to MM susceptibility in Anglo-Saxon populations (Bastiaens *et al.*, 2001; Valverde *et al.*, 1995) and also in the Northern French population (Matichard *et al.*, 2004). These variants also are related to red hair and fair skin (Demenais *et al.*, 2010). In Uruguay, these variants were present at frequencies similar to those of Spanish and French populations (Fernandez *et al.*, 2007; Ibarrola-Villava *et al.*, 2012b; Matichard *et al.*, 2004).

Our data suggest that being a carrier of non-synonymous changes is associated with a much higher risk of melanoma (Ibarrola-Villava *et al.*, 2012a; Ibarrola-Villava *et al.*, 2012b; Scherer *et al.*, 2009). The statistically significant associations for R160W and R151C are consistent with other results from Mediterranean populations (Hu *et al.*, 2014; Fagnoli *et al.*, 2006; Matichard *et al.*, 2004; Valverde *et al.*, 1995). However, in another Spanish population (from Valencia) the only RHC variants associated with statistically significantly increased risk of the disease were R160W and D294H (Scherer *et al.*, 2009).

The haplotype analysis allowed the detection of new genome structures in our samples: three haplotypes in the putative promoter region (TGA, CTG and TTG) that were not described in HapMap populations. The haplotypes TGG and CGG presented a very different frequency distribution to the ones present in Europe, whereas the haplotype frequencies for the MC1R gene region are close to the ones found in European or Spanish populations. These results suggest that the genome structure of the region gives support to? verifies the admixed structure of the Uruguayan population. The frequency spectrum of these haplotype blocks can arise by different mechanisms like admixture and recombination or genetic drift. But also, Uruguayan demographic history suggests a population expansion which could explain the observed haplotype frequencies (Bertoni *et al.*, 2005). The limited number of SNPs only allows to detect a protective haplotype. Moreover, the structure of the region around the MC1R gene seems to be labile to sequence changes. TUBB3 is a tubulin-beta 3 involved in neuronal dendrite formation (Katsetos *et al.*, 2003) at 2.5 kb from the MC1R gene. Recently, Dalziel *et al.* (2010) demonstrated an intergenic

splicing and alternative polyadenylation between these two genes that generate a chimeric protein. The expression level of the chimeric transcript MC1R-TUBB3 depends on concentration of the  $\alpha$ -MSH hormone. Whereas the normal MC1R transcript decreases, the chimeric transcript increases its expression with prolonged exposure to the hormone; a situation that resembles the responses to UV exposure (Dalziel *et al.*, 2010).

The relation between ancestry and the MC1R chromosome region could represent an important finding in our population to continue exploring by Admixture Mapping to detect new genes or variants involved in the development of sporadic melanoma (Chakraborty and Weiss, 1988; McKeigue, 1998), but also to describe new variants developed by the admixture structure itself (Zhu *et al.*, 2008).

## CONCLUSIONS

Our study provides, for the first time, information on melanoma, ancestry and MC1R gene variants for an admixed population from Latin America. In conclusion, European ancestry and the RHC variants, R151C and R160W, are risk factors for sporadic melanoma. However, further studies of the MC1R haplotype structure of the gene control region would help to clarify the interaction between admixture and the development of the disease.

## Abbreviations

AIM: ancestry informative marker; MM: malignant melanoma; MC1R: melanocortin 1 receptor; RHC: red hair colour; OR: odd ratio; SNP: single nucleotide polymorphism.

## BIBLIOGRAPHY

Acton R.T., Barton E.H., Hollowell W.W., Dreibelbis A.L., Go R.C.P., Barton J.C. (2004) Ancestry reported by white adults with cutaneous melanoma and control subjects in central Alabama. *BMC Cancer* 4: 47.

Bakos L., Masiero N.C.M.S., Bakos R.M., Burtet R.M., Wagner M.B., Benzano D. (2009) European ancestry and cutaneous melanoma in Southern Brazil. *J. Eur. Acad. Dermatol. Venereol.* 23: 304-7.

Bakos L., Wagner M., Bakos R.M., Leite C.S.M., Sperhake C.L., Dzekaniak K.S., Gleisner A.L.M. (2002) Sunburn, sunscreens, and phenotypes: some risk factors for cutaneous melanoma in southern Brazil. *Int. J. Dermatol.* 41: 557-62.

Barrett J.C., Fry B., Maller J., Daly M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-5.

Barrios E., Vasallo J., De Stéfani E. (2001) Patterns of cutaneous malignant melanoma incidence and mortality in Uruguay. *Melanoma Res.* 11 (suppl: S138).

Bastiaens M.T., ter Huurne J., Kielich C., Gruis N., Westendorp R.G., Vermeer B.J., Bavinck J.N. (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.

Bataille V. (2003) Genetic epidemiology of melanoma. *Eur. J. Cancer* 39: 1341-1347.

Bertoni B., Jin L., Chakraborty R., Sans M. (2005) Directional mating and a rapid male population expansion in a hybrid Uruguayan population. *Am. J. Hum. Biol.* 17: 801-8.

Bloethner S., Scherer D., Drechsel M., Hemminki K., Kumar R. (2009) Malignant melanoma: a genetic overview. *Actas Dermosifiliogr.* 100 Suppl: 38-51.

Bonilla C., Parra E.J., Pfaff C.L., Dios S., Marshall J.A., Hamman R.F., Ferrell R.E., Hoggart C.L., McKeigue P.M., Shriver M.D. (2004a) Admixture in the Hispanics of the San Luis Valley, Colorado, and its implications for complex trait gene mapping. *Ann. Hum. Genet.* 68: 139-153.

Bonilla C., Shriver M.D., Parra E.J., Jones A., Fernández J.R. (2004b) Ancestral proportions and their association with skin pigmentation and bone mineral density in Puerto Rican women from New York city. *Hum. Genet.* 115: 57-68.

Chakraborty R. (1985) Gene Identity in racial hybrids and estimation of admixture rates. In: Neel J.V., Ahuja Y.



- (Eds.) Genetic microdifferentiation in Man and others animals. Indian Anthropological Association, Delhi University, Anthropology Department: Delhi pp. 171-180.
- Chakraborty R., Weiss K.M. (1988) Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. *Proc. Natl. Acad. Sci. U.S.A.* 85: 9119-23.
- Dalziel M., Kolesnichenko M., das Neves R.P., Iborra E., Goding C., Furger A. (2010) -MSH regulates intergenic splicing of MC1R and TUBB3 in human melanocytes. *Nucleic Acids Res.* 1-15.
- Darvasi A., Shifman S. (2005) The beauty of admixture. *Nat. Genet.* 37: 118-119.
- Deménais F., Mohamdi H., Chaudru V., Goldstein A.M., Newton Bishop J.A., Bishop D.T., Kanetsky P.A., Hayward N.K., Gillanders E., Elder D.E., Avril M.F., Azizi E., van Belle P., Bergman W., Bianchi-Scarrà G., Bressac-de Paillerets B., Calista D., Carrera C., Hansson J., Harland M., Hogg D., Höiom V., Holland E.A., Ingvar C., Landi M.T., Lang J.M., Mackie R.M., Mann G.J., Ming M.E., Njauw C.J., Olsson H., Palmer J., Pastorino L., Puig S., Randerson-Moor J., Stark M., Tsao H., Tucker M.A., van der Velden P., Yang X.R., Gruis N. (2010) Association of MC1R Variants and Host Phenotypes With Melanoma Risk in CDKN2A Mutation Carriers: A GenoMEL Study. *J. Natl. Cancer Inst.* 102 (20): 1568-83.
- Duffy D.L., Box N.F., Chen W., Palmer J.S., Montgomery G.W., James M.R., Hayward N.K., Martin N.G., Sturm R.A. (2004) Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. *Hum. Mol. Genet.* 13: 447-61.
- Erdei E., Torres S.M. (2010) A new understanding in the epidemiology of melanoma. *Expert Rev. Anticancer Ther.* 10: 1811-1823.
- Fargnoli M.C., Spica T., Sera F., Pellacani G., Chiarugi A., Seidenari S., Carli P., Chimenti S., Peris K. (2006) Re: MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. *J. Natl. Cancer Inst.* 98: 144-5; author reply 145-6.
- Fernandez L., Milne R., Bravo J., Lopez J., Avilés J., Longo M., Benítez J., Lázaro P., Ribas G. (2007) MC1R: three novel variants identified in a malignant melanoma association study in the Spanish population. *Carcinogenesis* 28: 1659-64.
- Garbe C., Leiter U. (2009) Melanoma epidemiology and trends. *Clin. Dermatol.* 27: 3-9.
- Gerstenblith M.R., Goldstein A.M., Fargnoli M.C., Peris K., Ñ M.T.L. (2007) Comprehensive Evaluation of Allele Frequency Differences of MC1R Variants Across Populations. *Hum. Mutat.* 28: 495-505.
- Goldstein A.M., Chan M., Harland M., Hayward N.K., Deménais F., Bishop D.T., Azizi E., Bergman W., Bianchi-Scarra G., Bruno W., Calista D., Albright L.A.C., Chaudru V., Chompret A., Cuellar F., Elder D.E., Ghiorzo P., Gillanders E.M., Gruis N.A., Hansson J., Hogg D., Holland E.A., Kanetsky P.A., Kefford R.F., Landi M.T., Lang J., Leachman S.A., MacKie R.M., Magnusson V., Mann G.J., Bishop J.N., Palmer J.M., Puig S., Puig-Butille J.A., Stark M., Tsao H., Tucker M.A., Whitaker L., Yakobson E. (2007) Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J. Med. Genet.* 44: 99-106.
- Hidalgo P.C., Bengochea M., Abilleira D., Cabrera A., Alvarez I. (2005) Genetic Admixture Estimate in the Uruguayan Population Based on the Loci LDLR, GYP A, HBBG, GC and D7S8. *Int. J. Hum. Genet.* 5: 217-222.
- Hu H.H., Benfodda M., Dumaz N., Gazal S., Descamps V., Bourillon A., Basset-Seguín N., Riffault A., Ezzedine K., Bagot M., Bensussan A., Saiag P., Grandchamp B., Soufir N. (2014) A large French case-control study emphasizes the role of rare Mc1R variants in melanoma risk. *Biomed Res. Int.* 2014: 1-10.
- Ibarrola-Villava M., Fernandez L.P., Pita G., Bravo J., Floristan U., Sendagorta E., Feito M., Avilés J.A., Martin-Gonzalez M., Lázaro P., Benítez J., Ribas G. (2010) Genetic analysis of three important genes in

- pigmentation and melanoma susceptibility: CDKN2A, MC1R and HERC2/OCA2. *Exp. Dermatol.* 19: 836-44.
- Ibarrola-Villava M., Hu H.H., Guedj M., Fernandez L.P., Descamps V., Basset-Seguín N., Bagot M., Bensussan A., Saiag P., Fargnoli M.C., Peris K., Aviles J. A., Lluch A., Ribas G., Soufir N. (2012a) MC1R, SLC45A2 and TYR genetic variants involved in melanoma susceptibility in southern European populations: results from a meta-analysis. *Eur. J. Cancer* 48: 2183-91.
- Ibarrola-Villava M., Martin-Gonzalez M., Lazaro P., Pizarro A., Lluch A., Ribas G. (2012b) Role of glutathione S-transferases in melanoma susceptibility: association with GSTP1 rs1695 polymorphism. *Br. J. Dermatol.* 166: 1176-83.
- Kanetsky P.A., Ge F, Najarian D., Swoyer J., Panossian S., Schuchter L., Holmes R., Guerry D., Rebbeck T.R. (2004) Assessment of polymorphic variants in the melanocortin-1 receptor gene with cutaneous pigmentation using an evolutionary approach. *Cancer Epidemiol. Biomarkers Prev.* 13: 808-19.
- Kanetsky P.A., Swoyer J., Panossian S., Holmes R., Guerry D., Rebbeck T.R. (2002) A polymorphism in the agouti signaling protein gene is associated with human pigmentation. *Am. J. Hum. Genet.* 70: 770-5.
- Katsetos C.D., Herman M.M., Mörk S.J. (2003) Class III beta-tubulin in human development and cancer. *Cell Motil. Cytoskeleton* 55: 77-96.
- Landi M.T., Kanetsky P.A., Tsang S., Gold B., Munroe D., Rebbeck T., Swoyer J., Ter-Minassian M., Hedayati M., Grossman L., Goldstein A.M., Calista D., Pfeiffer R.M. (2005) MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. *J. Natl. Cancer Inst.* 97: 998-1007.
- Larre Borges A., Cuéllar F., Puig-Butillé J., Scarone M., Delgado L., Badenas C., Milà M., Malveyh J., Barquet V., Núñez J., Laporte M., Fernández G., Levrero P., Martínez-Asuaga M., Puig S. (2009) CDKN2A mutations in melanoma families from Uruguay. *Br. J. Dermatol.* 161: 536-41.
- Lascano A.R., Kuznitsky R., Cuestas E., Mainardi C., Albertini R., Borello A., Kurpis M., Campana R., Palazzo E. (2004) Factores de riesgo para melanoma cutáneo. Estudio de casos y controles en Córdoba, Argentina. *Medicina (Buenos Aires)* 64: 504-508.
- Luiz O.C., Gianini R.J., Gonçalves F.T., Francisco G., Festa-Neto C., Sanches J.A., Gattas G.J.F., Chammas R., Eluf-Neto J. (2012) Ethnicity and cutaneous melanoma in the city of Sao Paulo, Brazil: a case-control study. *PLoS One* 7: e36348.
- Matichard E., Verpillat P., Meziani R., Gérard B., Descamps V., Legroux E., Burnouf M., Bertrand G., Bouscarat F., Archimbaud A., Picard C., Ollivaud L., Basset-Seguín N., Kerob D., Lanténier G., Lebbe C., Crickx B., Grandchamp B., Soufir N. (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: 13e-13.
- McKeigue P.M. (1998) Mapping genes that underlie ethnic differences in disease risk: methods for detecting linkage in admixed populations, by conditioning on parental admixture. *Am. J. Hum. Genet.* 63: 241-51.
- Nikolaou V.A., Sypsa V., Stefanaki I., Gogas H., Papadopoulos O., Polydorou D., Plaka M., Tsoutsos D., Dimou A., Mourtzoukou E., Korfitis V., Hatzilou E., Antoniou C., Hatzakis A., Katsambas A., Stratigos A.J. (2008) Risk associations of melanoma in a Southern European population: results of a case/control study. *Cancer Causes Control* 19: 671-9.
- Palmer J.S., Duffy D.L., Box N.F., Aitken J.F., O’Gorman L.E., Green A.C., Hayward N.K., Martin N.G., Sturm R.A. (2000) Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? *Am. J. Hum. Genet.* 66: 176-86.
- Pimenta J.R., Zuccherato L.W., Debes A.A., Maselli L., Soares R.P., Moura-Neto R.S., Rocha J., Bydlowski S.P., Pena S.D.J. (2006) Color and genomic ancestry in Brazilians: a study with forensic microsatellites. *Hum. Hered.* 62: 190-5.

- Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A.R., Bender D., Maller J., Sklar P., de Bakker P.I.W., Daly M.J., Sham P.C. (2007) PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* 81: 559-575.
- Raimondi S., Sera F., Gandini S., Iodice S., Caini S., Maisonneuve P., Fargnoli M.C. (2008) MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int. J. Cancer* 122: 2753-60.
- Sans M., Salzano F.M., Chakraborty R. (1997) Historical genetics in Uruguay: Estimates of biological origins and their problems. *Hum. Biol.* 69: 161-170.
- Savage S.A., Gerstenblith M.R., Goldstein A.M., Mirabello L., Fargnoli M.C., Peris K., Landi M.T. (2008) Nucleotide diversity and population differentiation of the melanocortin 1 receptor gene, MC1R. *BMC Genet.* 9: 31.
- Scherer D., Kumar R. (2010) Genetics of pigmentation in skin cancer- A review. *Mutat. Res.* 705: 141-153.
- Scherer D., Nagore E., Bermejo J.L., Figl A., Botella-Estrada R., Thirumaran R.K., Angelini S., Hemminki K., Schadendorf D., Kumar R. (2009) Melanocortin receptor 1 variants and melanoma risk: a study of 2 European populations. *Int. J. Cancer* 125: 1868-75.
- Schmerling R.A., Loria D., Cinat G., Ramos W.E., Cardona A.F., Sánchez J.L., Martínez-Said H., Buzaid A.C. (2011) Cutaneous melanoma in Latin America: the need for more data. *Rev. Panam. Salud Pública* 30: 431-8.
- Solé X., Guinó E., Valls J., Iñesta R., Moreno V. (2006) SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22: 1928-9.
- Sortino-Rachou A.M., Curado M.P. (2011) Cutaneous melanoma in Latin America: a population-based descriptive study- Melanoma cutâneo na América Latina: estudo descritivo de base populacional. *Cancer* 27: 565-572.
- Stratigos A.J., Dimisianos G., Nikolaou V., Poulou M., Sypsa V., Stefanaki I., Papadopoulos O., Polydorou D., Plaka M., Christofidou E., Gogas H., Tsoutsos D., Kastana O., Antoniou C., Hatzakis A., Kanavakis E., Katsambas A.D. (2006) Melanocortin receptor-1 gene polymorphisms and the risk of cutaneous melanoma in a low-risk southern European population. *J. Invest. Dermatol.* 126: 1842-9.
- Suarez-Kurtz G., Vargens D.D., Struchiner C.J., Bastos-Rodrigues L., Pena S.D.J. (2007) Self-reported skin color, genomic ancestry and the distribution of GST polymorphisms. *Pharmacogenet. Genomics* 17: 765-71.
- Valverde P., Healy E., Jackson I., Rees J.L., Thody A.J. (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* 11: 328-30.
- Wong T.H., Rees J.L. (2005) The relation between melanocortin 1 receptor (MC1R) variation and the generation of phenotypic diversity in the cutaneous response to ultraviolet radiation. *Peptides* 26: 1965-71.
- Zhu X., Tang H., Risch N. (2008) Admixture Mapping and the Role of Population Structure for Localizing Disease Genes. *Adv. Genet.* 60: 547-569.

## ACKNOWLEDGEMENTS

We are grateful to all the participants of the study, and Clínica de Lesiones Pigmentarias of the Hospital “Manuel Quintela”. We thank P. Silveira from the Laboratorio de Análisis Clínicos and M. Gardner for her review of manuscript language. This study was supported by grants from the Comisión Honoraria de Lucha contra el Cáncer (CHLCC), Agencia Nacional de Innovación e Investigación (ANII) and Programa de Desarrollo de las Ciencias Básicas (PEDECIBA) and partially supported by a grant from the Ministerio de Salud Carlos III (PI10-00405, CP08-00069 to G.R.).

### Authors' contributions

BB and MMA conceived and planned the investigation. JH and BB wrote the manuscript; JH and VC performed the statistical analysis; JP, SN and ALB recruited sporadic melanoma cases and controls and performed the clinical examination; JH, GR and MC processed blood samples and/or genotyped the SNPs. All authors read and approved the final manuscript.

Competing interests: The authors have no competing interests