Official journal of the American College of Medical Genetics and Genomics **ORIGINAL RESEARCH ARTICLE** Genetics inMedicine

Open

Characterization of individuals at high risk of developing melanoma in Latin America: bases for genetic counseling in melanoma

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Purpose: *CDKN2A* is the main high-risk melanoma-susceptibility gene, but it has been poorly assessed in Latin America. We sought to analyze *CDKN2A* and *MC1R* in patients from Latin America with familial and sporadic multiple primary melanoma (SMP) and compare the data with those for patients from Spain to establish bases for melanoma genetic counseling in Latin America.

Methods: *CDKN2A* and *MC1R* were sequenced in 186 Latin American patients from Argentina, Brazil, Chile, Mexico, and Uruguay, and in 904 Spanish patients. Clinical and phenotypic data were obtained.

Results: Overall, 24 and 14% of melanoma-prone families in Latin America and Spain, respectively, had mutations in *CDKN2A*. Latin American families had *CDKN2A* mutations more frequently ($P = 0.014$) than Spanish ones. Of patients with SMP, 10% of those from Latin America and 8.5% of those from Spain had mutations in *CDKN2A* ($P = 0.623$). The most recurrent *CDKN2A* mutations were c.-34G>T and p.G101W. Latin American patients had fairer hair ($P = 0.016$) and skin ($P < 0.001$) and a higher prevalence of *MC1R* variants ($P = 0.003$) compared with Spanish patients.

Conclusion: The inclusion criteria for genetic counseling of melanoma in Latin America may be the same criteria used in Spain, as suggested in areas with low to medium incidence, SMP with at least two melanomas, or families with at least two cases among first- or second-degree relatives.

Genet Med advance online publication 17 December 2015

Key Words: *CDKN2A*; familial; Latin America; melanoma; *MC1R*

INTRODUCTION

Melanoma is the most aggressive of common skin cancers because of its tendency to metastasize. Its incidence is rapidly increasing, especially among Caucasian populations. Melanoma is the second most diagnosed cancer among patients younger than 30 years of age,[1] and the 3-year survival rate for patients

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Submitted 13 July 2015; accepted 29 September 2015; advance online publication 17 December 2015. doi:10.1038/gim.2015.160

with metastases is around 15%.[2] Identification of individuals at high risk of developing melanoma is necessary since an early diagnosis improves the disease prognosis.[3]

Melanoma is caused by the interaction of environmental, phenotypic, and genetic factors. The main environmental risk factor for melanoma is sun exposure.[4] Individuals with fair skin, red hair, and/or a high nevi count have an increased risk of developing melanoma.[5] To date, *CDKN2A*, which encodes the tumor suppressor proteins p16INK4A and p14ARF, is the major high-risk gene involved in melanoma susceptibility.[6] *CDKN2A* has been widely studied in melanoma patients from the United States, Europe, and Australia.[6] The frequency of germline mutations in *CDKN2A* varies across populations (5–72%) and depends on the selection criteria used.[6,7] Haplotype analysis indicates a founder effect for most of the recurrent mutations detected.[8] Identification of the prevalence of *CDKN2A* mutations in patients at high risk for melanoma and the correlation of these mutations with clinical data has been crucial for establishing genetic counseling for melanoma. Melanoma risk may also be modulated by common genetic variants acting as low- to medium-penetrance variants.[9] *MC1R* plays a key role in pigmentation and is responsible for phenotypic characteristics such as hair and skin color and the capacity of response to ultraviolet radiation.[10] Several *MC1R* variants are associated with a moderately increased melanoma risk and also modulate the effect of *CDKN2A* mutations in carriers.[11]

Genetic counseling and specific dermatological follow-up may be offered to patients at high risk for melanoma.[12] In countries with a low to medium incidence of melanoma, genetic counseling is offered to patients with two primary melanomas and/or to families with two melanoma cases and/or one pancreatic adenocarcinoma and one melanoma in first- or second-degree relatives (the "rule of two"). In countries with a moderate to high incidence of melanoma, however, genetic counseling is offered to patients with three primary melanomas and to families with three cases of melanoma or pancreatic cancer in first- or second-degree relatives (the "rule of three").[13] It has been demonstrated that melanoma genetic counseling has a positive impact on the improvement of total body skin examination and self-examination of the skin in unaffected individuals carrying germline mutations after test reporting, whereas affected carriers maintain high levels of screening adherence.[14] Furthermore, after melanoma genetic counseling, unaffected members of high-risk melanoma families report improvements in daily routine sun protection, showing that genetic counseling may motivate sustained improvements in prevention behaviors.[15] Thus it is very important for both melanoma patients and unaffected individuals from the family to be included in genetic counseling programs.

Few studies have assessed the prevalence of *CDKN2A* mutations or *MC1R* variants and phenotypic characteristics in patients at high risk for melanoma from Latin American countries. *CDKN2A* mutations have been identified in 13.6% of melanoma-prone families from São Paulo, Brazil,[16] whereas one study reported no mutations in Porto Alegre,[17] and in a different cohort the mutation frequency was 7%.[18] In melanoma-prone families from Uruguay, 5/6 families had *CDKN2A* mutations.[19] Phenotypic and genetic characterization of individuals at high risk for melanoma from Latin America may improve their management and implement genetic counseling in these countries. We present the molecular characterization of *CDKN2A* and *MC1R* genes in the largest set of patients at high risk for melanoma from distinct Latin American countries (Argentina, Brazil, Chile, Mexico, and Uruguay), and we compare the data with two sets of Spanish patients at high risk for melanoma to establish bases for genetic counseling in Latin America.

MATERIALS AND METHODS

The multicenter cross-sectional study included 1,090 patients at high risk for melanoma: 758 patients with familial melanoma (FM) and 332 patients with SMP from Latin American countries and Spain. Because Latin America is a region with a low incidence of melanoma (GLOBOCAN 2012, World Health Organization; http://globocan.iarc.fr), the inclusion criteria followed the rule of two.

Overall, 186 Latin American melanoma patients were recruited from Argentina (n = 10), Chile (n = 28), Mexico (n = 6), Uruguay (n = 25), and Brazil (n = 117), which included two sets of patients: Porto Alegre (Southern Brazil) (n = 58) and São Paulo (southeast region) (n = 59). The contribution of each country to the study resulted in a broad representation of a number of Latin American countries. A set of 904 Spanish patients with melanoma from Barcelona (n = 706) and Valencia (n = 198) also were included using the same selection criteria.

The number of primary melanomas, age at diagnosis, number of melanoma cases in the family, ancestral origin, and phenotypic data (hair and eye color, skin phototype, and nevi count) were recorded by dermatologists for most of the patients. Although the number of missing values was higher in the set of Spanish patients than in the Latin American patients, this did not introduce a bias, and the information recruited was informative for the whole cohort: Spanish patients were recruited consecutively, and missing data were distributed randomly; two different cohorts from Spain where used to minimize the bias due to the data collection procedure; and the variable with the greatest amount of missing data had information from at least 600 Spanish patients. Partial genetic information of the patients with melanoma from Spain and Brazil, and a subset of pedigrees from Uruguay, has been previously reported.[16–21]

The study was approved by the ethical committee of the Hospital Clinic of Barcelona. The patients gave their written, informed consent.

CDKN2A and *MC1R* molecular screening

Molecular characterization of *CDKN2A* was performed in all patients. *CDKN2A* was sequenced in all patients, as previously described.[16,18,20,21] *MC1R* was sequenced as described elsewhere.[22,23] The *MC1R* genotype was available from all patients from Argentina and Chile, 57% (33/58) patients from Porto Alegre, Brazil, 92% (54/59) patients from São Paulo, Brazil, 96% (24/25)

patients from Uruguay, 59% (419/706) patients from Barcelona, Spain, and 94% (186/198) patients from Valencia, Spain. *MC1R* genotype data were not available for patients from Mexico.

Statistical analyses

For the statistical analyses, the most common *MC1R* variants were classified as r variants (not associated with red hair color: p.V60L, p.V92M, p.R163Q) or R variants (associated with red hair color: p.D84E, p.R142H, p.R151C, p.I155T, p.R160W, p.D294H).[10]

SPSS software version 17.0 (IBM, Chicago, IL) was used. Two-sided Pearson χ^2 or Fisher exact tests were used for categorical variables, as applicable. Student's *t*-test was used for quantitative variables. Adjusted *P* values were calculated using the Bonferroni correction. The test was considered significant if the *P* value or adjusted *P* value (as applicable) was <0.05.

RESULTS

The study included a set of 1,090 patients with melanoma from distinct Latin American countries and Spain. Latin America and Spain had similar frequencies of FM cases (67.7 and 69.9%, respectively) and SMP (32.3 and 30.1%, respectively; $P = 0.600$), and there were no gender differences (40.3% male and 58.7% female vs. 41.5% male and 58.5% female, respectively; $P = 0.806$). Since Latin America is a mixed population from European, Native, African and Asian origin as a result of the colonization process and migratory effects,[24] we collected information regarding the patients' ancestral origin. The four grandparents of more than 70% of Latin American patients were of European origin. Latin American and Spanish patients differed in pigmentation traits. Latin American patients had fairer hair color (adjusted $P = 0.016$) and skin phototype (adjusted $P < 0.001$) than Spanish patients. No differences were observed for nevi count or eye color (**Table 1**).

Considering all patients, *CDKN2A* mutation prevalence was 19% in Latin America and 12% in Spain. *CDKN2A* mutation frequency in SMP was similar in Latin America (10%) and Spain (8.5%) ($P = 0.623$). However, the prevalence of *CDKN2A* mutations in Latin American melanoma-prone families was higher than in Spain (24 and 14%, respectively; $P = 0.019$). The frequency of mutations varied among countries. Whereas southern Brazil had a low mutation prevalence, Chile and Uruguay showed a high prevalence of mutations in both SMP and FM (**Table 2**).

The *CDKN2A* mutations differed in each country (**Table 3**). Overall, 74% (23/31) of Latin American *CDKN2A* mutation carriers had a mutation also found in Spanish patients with melanoma. The most prevalent mutations in Latin America (c.-34G>T and p.G101W (c.301G>T)) were among the most recurrent mutations in Spain, which are p.G101W (33%), p.V59G (c.176T>G) (7%), c.-34G>T (6%), p.A36RfsX17 (c.106delG) (6%), and p.E120fsX145 (c.358delG) (5%) (**Table 3**). Mutation c.-34G>T was present in 90% of families from Chile, and families from São Paulo (Brazil) and Uruguay with *CDKN2A* mutations. Mutation p.G101W was present in families from Argentina, São Paulo (Brazil), and Uruguay. The other mutations detected in Latin America were restricted to a few pedigrees.

CDKN2A mutations have been previously associated with a lower age at diagnosis, number of primary melanomas, and the number of cases in the family.[6] The whole set of patients also showed these associations (**Table 4**). Latin American patients with melanoma carrying a *CDKN2A* mutation had an increased number of cases in the family and a lower age at diagnosis, but the number of personal primary melanomas did not reach significance.

We sequenced *MC1R* to assess the distribution of *MC1R* variants across countries (**Table 5**). We observed differences in the number and type of variants between Latin America and Spain. We detected *MC1R* variants in 80.5% of Latin American and 67.9% of Spanish patients ($P = 0.003$), with a similar R variant frequency (39.6 vs. 36.3%, respectively; $P = 0.514$) but a higher r variant prevalence in Latin America (40.9 vs. 31.6%, respectively; $P = 0.033$). We analyzed the frequencies of the most common R and r variants, comparing Latin America and Spain (**Supplementary Table S1** online). When adjusting using the Bonferroni correction, we found a significantly increased presence of p.R160W (17.4 vs. 7.5%; adjusted $P < 0.005$) and p.R163Q (14.1 vs. 5.2%; adjusted $P < 0.005$) in Latin America, but we should take into consideration that all patients carrying the p.R163Q variant in this study were from only three study sites: Brazil (São Paulo), Chile, or Uruguay. The p.D294H variant was more frequent in Spain (5.4 vs. 13.3%; adjusted $P = 0.045$). The presence of *MC1R* variants and R variants correlated with phenotype (**Supplementary Tables S2 and S3** online).

DISCUSSION

Latin America has a low incidence of melanoma (GLOBOCAN 2012). The characterization of melanoma genes has allowed other areas with low to medium incidence of melanoma, such as Spain, to recommend genetic counseling for patients with melanoma.[12,25] To date, only a few specialized centers in Latin America offer melanoma genetic counseling, and there is little knowledge of the implication of high-risk genes in melanoma susceptibility. This study presents the clinical and molecular characterization of *CDKN2A* and *MC1R* in the largest set of Latin American patients at high risk for melanoma.

CDKN2A mutation frequency in melanoma-prone families was higher in Latin America than Spain, using the same selection criteria. By contrast, both areas had similar SMP *CDKN2A* mutation prevalence, consistent with that reported in other studies (8.2–9%).[25,26] The age at diagnosis and number of primary melanomas were associated with the presence of mutations in *CDKN2A*, as previously reported.[6] Otherwise, we did not find associations between *CDKN2A* mutation and nevi count, suggesting that other genes could play a role in nevogenesis.[27,28] Most *CDKN2A* mutations identified had been previously detected in European or North American patients with melanoma. The most prevalent mutation in Latin America was c.-34G>T. This mutation occurs at a high

Table 1 Characteristics and phenotypic data of patients with melanoma, by country (region)

	Argentina		Brazil (Porto Alegre)		Brazil (São Paulo)		Chile		Mexico		Uruguay		Spain (Barcelona)		Spain (Valencia)		Latin America		Spain		Adjusted	Total	
	n	%	*n*	%	*N*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*P* value[a]	*N*	%
Patients with melanoma																							
FM[b]	5	50.0	45	77.6	37	62.7	16	57.1	3	50.0	20	80.0	472	66.9	160	80.8	126	67.7	632	69.9	—	758	69.5
SMP	5	50.0	13	22.4	22	37.3	12	42.9	3	50.0	5	20.0	234	33.1	38	19.2	60	32.3	272	30.1		332	30.5
Total	10		58		59		28		6		25		706		198		186		904			1,090	
Phenotypic characteristics																							
Hair color																							
Red	1	10.0	5	14.7	2	3.4	1	3.6	1	16.7	4	19.0	30	5.8	16	9.4	14	8.9	46	6.7	**0.016**	60	7.1
Blond	2	20.0	17	50.0	26	44.1	8	28.6	1	16.7	2	9.5	126	24.4	37	21.8	56	35.4	163	23.7		219	25.9
Dark	7	70.0	12	35.3	31	52.5	19	67.8	4	66.7	15	71.4	361	69.8	117	68.8	88	55.7	478	69.6		566	67.0
Missing	0		24		0		0		0		4		189		28		28		217			245	
Total	10		58		59		28		6		25		706		198		186		904			1,090	
Eye color																							
Fair	5	50.0	20	58.8	27	45.8	14	50.0	2	33.3	9	42.9	226	43.9	52	29.9	77	48.7	278	40.3	0.240	355	41.9
Dark	5	50.0	14	41.2	32	54.2	14	50.0	4	66.7	12	57.1	289	56.1	122	70.1	81	51.3	411	59.7		492	58.1
Missing	0		24		0		0		0		4		191		24		28		215			243	
Total	10		58		59		28		6		25		706		198		186		904			1,090	
Skin color[c]																							
Fair	7	70.0	56	98.2	51	86.4	21	75.0	3	50.0	18	85.7	299	55.2	75	42.1	156	86.2	374	51.9	**<0.001**	530	58.8
Dark	3	30.0	1	1.8	8	13.6	7	25.0	3	50.0	3	14.3	243	44.8	103	57.9	25	13.8	346	48.1		371	41.2
Missing	0		1		0		0		0		4		164		20		5		184			189	
Total	10		58		59		28		6		25		706		198		186		904			1,090	
Nevi count																							
<50	—		11	47.8	38	64.4	9	32.1	4	66.7	12	63.2	195	38.7	72	73.5	74	54.8	267	44.4	0.332	341	46.3
50—100	—		3	13.0	10	16.9	9	32.1	1	16.7	3	15.8	130	25.8	21	21.4	26	19.3	151	25.1		177	24.0
>100	—		9	39.1	11	18.6	10	35.7	1	16.7	4	21.1	179	35.5	5	5.1	35	24.9	184	30.6		219	29.7
Missing	10		35		0		0		0		6		202		100		51		302			353	
Total	10		58		59		28		6		25		706		198		186		904			1,090	
Ancestral origin[d]																							
European	8	88.9	44	78.6	35	59.3	20	74.1	4	66.6	20	80.0	—	—	—	—	131	73.2	—	—	—	—	—
Latin	1	11.1	12	21.4	20	33.9	5	18.5	1	16.7	1	20.0	—	—	—	—	40	22.3	—	—		—	—
Amerindian	0	0	0	0	0	0	2	7.4	1	16.7	0	0	—	—	—	—	3	1.7	—	—		—	—
African American	0	0	0	0	4	6.8	0	0	0	0	1	20.0	—	—	—	—	5	2.8	—	—		—	—
Missing	1		2		0		1		0		3		—	—	—	—	7		—	—		—	—
Total	10		58		59		28		6		25		—	—	—	—	186		—	—		—	—

Statistically significant *P* values are given in bold.

SMP, sporadic multiple primary melanoma.

[a]*P* values were obtained comparing Latin America with Spain. Bonferroni correction was used to obtain the adjusted *P* values. [b]In the familial melanoma (FM) category we included families with at least two melanoma cases.. [c]Skin color was classified according to the Fitzpatrick phototype classification: fair (phototypes I or II) and dark (phototypes III–V). [d]The ancestral origin of the Latin American patients with melanoma is indicated according to the origin of their grandparents. "European" means all grandparents were born in a European country. "Latin," "Amerindian," and "African American" indicate that at least one of the grandparents was born in Latin America (but had Spanish or Portuguese ancestors), was a descendent of natives, or had an African-American origin, respectively. In the case that the grandparents were of different origins, for example, African American and Amerindian, the ancestral origin was indicated taking into consideration the darkest skin color: African American/Amerindian/Latin. There were no individuals with Asian origin.

Table 2 *CDKN2A* mutation distribution between families according to the number of melanoma cases by country (region)

Country (region)	All pedigrees included (*N*)	Patients with SMP					All melanoma-prone families					Families with two cases				Families with three cases				Families with four or more cases				
		Total		*CDKN2A* mutation		*P* value	Total		*CDKN2A* mutation		*P* value	Total		*CDKN2A* mutation		Total		*CDKN2A* mutation		Total		*CDKN2A* mutation		
		n	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*P* value[b]
Argentina	10	5	50.0	1	20.0		5	50.0	0	0		4	80.0	0	0	1	20.0	0	0	0	0	0	0	
Brazil (Porto Alegre)	50	13	26.0	0	0		37	74.0	3	8.1		27	73.0	1	3.7	7	18.9	1	14.3	3	8.1	1	33.3	
Brazil (São Paulo)	57	22	38.6	1	3.6		35	61.4	8	22.4		21	60.0	1	4.8	13	37.1	7	53.8	1	2.9	0	0	
Chile	27	12	44.4	3	25.0		15	55.6	7	46.7		12	80.0	5	41.7	1	6.7	0	0	2	13.3	2	100	
Mexico	5	3	60.0	0	0		2	40.0	1	50.0		2	100	1	50.0	0	0	0	0	0	0	0	0	
Uruguay	20	5	25.0	1	20.0		15	75.0	8	40.0		11	73.3	4	36.4	4	26.7	3	75.0	0	0	0	0	
Spain (Barcelona)	564	234	41.5	20	8.5		330	58.5	47	14.2		269	81.5	30	11.2	47	14.2	11	23.4	14	4.2	6	42.9	
Spain (Valencia)	147	38	25.9	3	7.9		109	74.1	15	13.8		79	72.5	8	10.1	22	20.2	5	22.7	8	7.3	2	25.0	
Missing	0	0	—	0	—		0	—	0	—		0	—	0	—	0	—	0	—	0	—	0	—	
Latin America	169	60	35.5	6	10.0	0.623	109	64.5	26	23.9	**0.019**	77	70.6	12	15.6	26	23.9	11	42.3	6	5.5	3	50.0	**0.007**
Spain	711	272	38.3	23	8.5		439	61.7	62	14.1		348	79.3	38	10.9	69	15.7	16	23.2	22	5.0	8	36.4	**<0.001**
Total	880	332	37.7	29	8.9		548	62.3	88	16.1		425	77.6	50	11.8	95	17.3	27	28.4	28	5.1	11	39.3	**<0.001**

Statistically significant *P* values are given in bold.

[a]*P* values to assess differences in mutation frequency among families with sporadic multiple primary melanoma (SPM) and among all melanoma-prone families were obtained by comparing the global result of Latin America versus Spain. [b]*P* values to assess differences in mutation frequency among families according to the number of melanoma cases were assessed separately in Latin America, in Spain, and in the entire set of patients (total).

Table 3 *CDKN2A* genetic results

Exon	Protein change		Argentina		Brazil (Porto Alegre)		Brazil (São Paulo)		Chile		Mexico		Uruguay		Spain (Barcelona)		Spain (Valencia)		Latin America		Spain		Total	
cDNA change	p14ARF	p16INK4A	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*N*	%
1β																								
c.127G>C	p.V43L	—	0	0	0	0	1	11.1	0	0	0	0	0	0	0	0	0	0	1	3.4	0	0	1	0.9
1α																								
c.-34G>T	—	—	0	0	1	33.3	3	33.3	9	90	0	0	1	12.5	5	7.5	0	0	14	45.2	5	5.9	19	16.2
c.106delG	—	p.A36RfsX17	0	0	0	0	0	0	0	0	0	0	0	0	5	7.5	0	0	0	0	5	5.9	5	4.2
c.142C>A	—	p.P48T	0	0	1	33.3	3	33.3	0	0	0	0	0	0	0	0	0	0	4	12.9	0	0	4	3.4
c.146T>C	—	p.I49T	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1	3.2	0	0	1	0.9
2																								
c.159G>C	p.D68H	p.M53I	0	0	1	33.3	0	0	0	0	0	0	0	0	0	0	0	0	1	3.2	0	0	1	0.9
c.176T>G	p.S73R	p.V59G	0	0	0	0	0	0	0	0	0	0	0	0	2	3.0	4	22.2	0	0	6	7.0	6	5.1
c.262G>T	p.G102V	p.E88X	0	0	0	0	0	0	0	0	0	0	2	25.0	2	3.0	0	0	2	6.5	2	2.4	4	3.4
c.301G>T	p.R115L	p.G101W	1	100	0	0	1	11.1	0	0	0	0	5	62.5	24	35.8	4	22.2	7	22.6	28	32.9	35	29.9
c.358delG	—	p.E120fsX145	0	0	0	0	0	0	0	0	0	0	0	0	1	1.5	3	16.7	0	0	4	4.7	4	3.4
c.430C>T	—	p.R144C	0	0	0	0	0	0	1	10	0	0	0	0	0	0	0	0	1	3.2	0	0	1	0.9
3																								
IVS2-105A>G	—	—	0	0	0	0	1	11.1	0	0	0	0	0	0	0	0	0	0	1	3.2	0	0	1	0.9
All exons	Other[a]	Other[a]	0	0	0	0	0	0	0	0	0	0	0	0	28	41.7	7	38.9	0	0	35	41.2	35	29.9
Total			1		3		9		10		1		8		67		18		31		85		117	
		p16INK4A Polymorphism																						
		p.A148T																						
		Yes	—	—	7	12.1	5	8.6	1	4.0	—	—	3	12	65	9.2	15	7.6	16	9.6	80	8.9	96	9.0
		No	—	—	51	87.9	53	91.4	24	96.0	—	—	22	88	638	90.8	183	92.4	150	90.4	821	91.1	971	91.0
		Missing	10		0		1		3		6		0		3		0		20		3		23	
		Total	10		58		59		28		6		25		706		198		186		904		1,090	

There were no statistical differences between the prevalence of the p.A148T polymorphism among melanoma patients in Latin America and Spain ($P = 0.768$).

[a]The other *CDKN2A* mutations identified in the Spanish population affecting only p14ARF were p.R21RfsX46 (c.60ins16), p.G32R (c.94G>A), and p.A121T (c.318G>A); those affecting p16INK4A were p.A5T (c.13G>A), p.P11T (c.31C>A), p.G35E (c.104G>A), p.N39S (c.116A>G), p.Y44X (c.131dup), p.Q50R (c.149A>G), p.G55V (c.164G>T), p.L65P (c.194T>C), p.N71S (c.212A>G), p.R80X (c.238C>T), p.P81S (c.241C>T), p.D84Y (c.250G>T), p.R87W (c.259C>T), p.R99W (c.295C>T), p.G101R (c.301G>A), p.A102V (c.305C>T), p.R112P (c.335G>C), p.E120SfsX21 (c.359_365del), p.G122R (c.364G>C), p.A127S (c.379G>A), and p.D153N (c.457G>A).

Table 4 Clinical and phenotypic characteristics of melanoma patients according to the presence of a *CDKN2A* mutation, by country

	Argentina		Brazil (Porto Alegre)		Brazil (Sao Paulo)		Chile		Mexico		Uruguay		Spain (Barcelona)		Spain (Valencia)		Latin America		Adj.	Spain			Total		
	n	%	*n*	%	*N*	%	*N*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*P*	*N*	%	Adj. *P*	*N*	%	Adj. *P*
CDKN2A mutation carriers																									
Hair color																									
Red	0	0	0	0	1	50.0	0	0	0	0	3	75.0	7	23.3	2	12.5	4	28.6	**0.025**	9	19.6	**0.015**	13	21.7	**<0.002**
Blond	0	0	0	0	2	7.7	3	37.5	0	0	0	0	9	7.1	2	5.4	5	8.9		11	6.7		16	7.3	
Dark	1	14.3	1	8.3	9	29.0	7	36.8	2	50.0	7	46.7	65	18.0	13	11.1	27	30.7		78	16.3		105		
Missing	0		2	—	0		0		0		1		20		2		3			22			25		
Total	1		3	—	12		10		2		11		101		19		39			120			159		
Eye color																									
Fair	0	0	1	5.0	5	18.5	4	28.6	0	0	4	44.4	31	13.7	6	11.5	14	18.2	0.950	37	13.3	1.000	51	14.4	1.000
Dark	1	20.0	0	0	7	21.9	6	42.9	2	50.0	6	50.0	49	17.0	11	9	22	27.2		60	14.6		82	16.7	
Missing	0		2		0		0		0		1		21		2		3			23			26		
Total	1		3		12		10		2		11		101		19		39			120			159		
Skin color[a]																									
Fair	1	14.3	3	5.4	11	21.6	9	42.9	1	33.3	10	55.6	46	15.4	4	5.3	35	22.4	1.000	50	13.4	1.000	85	16.0	1.000
Dark	0	0	0	0	1	12.5	1	14.3	1	33.3	0	0	35	14.4	15	14.6	3	12.0		50	14.5		53	14.3	
Missing	0		0		0		0		0		1		20		0		1			20			21		
Total	1		3		12		10		2		11		101		19		39			120			159		
Nevi count					0		0		0																
<50	—	—	0	0	10	26.3	2	22.2	1	25.0	8	66.7	26	13.3	8	11.1	21	28.4	0.765	34	12.7	0.260	55	16.1	1.000
50—100	—	—	0	0	1	10.0	1	11.1	1	100	0	0	31	23.7	3	14.3	3	11.5		33	21.9		36	20.3	
>100	—	—	1	11.1	1	9.1	7	70.0	0	0	2	50.0	29	16.2	0	0	11	31.4		29	15.8		40	18.3	
Missing	—	—	2		0		0		0		1		16		8		4			24			28		
Total	—	—	3		12		10		2		11		102		19		39			120			159		
No. of MMs																									
1	0	0	1	3.7	4	16.7	5	35.7	1	100	1	100	43	12.3	13	9.5	12	16.7	1.000	56	11.5	**<0.002**	68	12.2	**<0.002**
2	1	25.0	1	5.9	5	22.7	4	36.4	0	0	3	42.9	33	12.3	3	6.1	14	22.2		36	11.4		50	13.2	
3	0	0	1	10.0	1	14.3	1	33.3	1	33.3	0	0	12	22.2	2	25	4	16.7		14	22.6		18	20.9	
≥4	0	0	0	0	2	33.3	0	0	0	0	3	100	12	48	1	25	5	38.5		13	44.8		18	42.9	
Missing	0		0		0		0		0		4		1		0		4			1			5		
Total	1		3		12		10		2		11		102		19		39			120			159		
No. of A148T carriers with melanoma																									
1	—	—	5	16.1	1	3.4	0	0	—	—	0	0	33	9.5	8	5.8	6	8.1	1.000	41	8.4	1.000	47	8.4	1.000
2	—	—	1	5.9	4	19.0	0	0	—	—	1	14.3	24	9.0	4	8.2	6	10.9		28	8.9		34	9.2	
≥3	—	—	2	14.3	0	0	1	33.3	—	—	0	0	7	9.0	3	25.0	3	9.1		10	11.1		13	10.6	
Missing	—	—	0		0		0		—	—	2		1		0		2			1			3		
Total	—	—	8		5		1		—	—	3		65		15		17			80			97		
Age at diagnosis of first melanoma[b]	Years	SD	Years	SD	Years	SD	Years	SD	Years	SD	Years	SD	Years	SD	Years	SD	Years	SD	*P* value	Years	SD	*P* value	Years	SD	*P* value
CDKN2A mutation	39.0	—	40.3	18.0	43.7	10.6	43.4	9.8	47.5	3.5	36.3	15.0	39.4	12.7	42.7	15.4	41.5	11.9	**0.006**	40.0	13.2	**<0.001**	40.4	12.8	**<0.001**
CDKN2A wild type	44.9	13.6	50.0	13.6	45.7	13.2	52.6	16.4	39.2	6.8	51.8	17.3	47.2	16.6	47.8	16.8	48.5	13.8		47.4	16.6		47.5	16.2	
Total	44.3	12.9	49.5	13.8	45.3	12.6	49.2	14.8	42.0	6.9	44.1	17.7	46.1	16.2	47.3	16.7	47.1	13.7		46.4	16.4		46.5	16.0	

P values were obtained by comparing carriers vs. noncarriers individually in Latin America, Spain, and the total sample. Adjusted *P* values were calculated using the Bonferroni correction. Statistically significant *P* values are given in bold.

MM, primary melanoma.

[a]Skin color was classified according to the Fitzpatrick phototype classification: fair (phototypes I or II) and dark (phototypes III–V). [b]The age at diagnosis of first melanoma was not available in 1/28 (3.4%) of Chilean, 2/20 (10%) of Uruguayan, 64/707 (9.1%) of Barcelonan, and 3/195 (1.5%) of Valencian patients.

Table 5 *MC1R* variant distribution

	Argentina		Brazil (Porto Alegre)		Brazil (São Paulo)		Chile		Mexico		Uruguay		Spain (Barcelona)		Spain (Valencia)		Latin America		Spain		*P* value	Total	
	n	%	*n*	%	*N*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%		*N*	%
MC1R WT	1	10.0	10	30.3	9	16.7	6	21.4	—	—	3	12.5	118	28.2	75	41.2	29	19.5	193	32.1	**0.003**	222	29.6
≥1 *MC1R* variant[a]	9	90.0	23	69.7	45	83.3	22	78.6	—	—	21	87.5	301	71.8	107	58.8	120	80.5	408	67.9		528	70.4
R/R, R/r, or R/WT	4	40.0	10	30.3	27	50.0	7	25.0	—	—	11	45.8	168	40.1	50	27.5	59	39.6	218	36.3	0.507	277	36.9
r/r or r/WT	5	50.0	13	46.4	18	33.3	15	53.6	—	—	10	41.7	133	31.7	57	31.3	61	40.9	190	31.6	**0.033**	253	33.4
Missing	0		25		5		0		6		1		287		12		37		303			340	
Total	10		58		59		28		6		25		706		198		186		904			1,090	

P values were obtained comparing Latin America versus Spain. Statistically significant *P* values are given in bold.

R, *MC1R* variant associated with the red hair color phenotype (p.D84E, p.R142H, p.R151C, p.I155T, p.R160W, p.D294H, and rare frameshift variants); r, *MC1R* variants not associated with the red hair color phenotype (p.V60L, p.V92M, p.R163Q, and other rare missense variants); WT, wild type. [a]Synonymous variants were considered *MC1R* WT; all other missense or frameshift nucleotide changes, either prevalent or rare, were considered *MC1R* variants.

frequency among unrelated families from Chile, suggesting a possible founder effect. In one family from Chile we detected p.R144C (c.430C>T), previously detected at the germline level in a patient with pancreatic cancer.[29] Mutation p.G101W is also frequent in Latin America, as in Mediterranean countries (Italy, France, and Spain)[7] where haplotype analysis showed a founder effect.[30] We identified four other mutations in Brazil: p.P48T (c.142C>A), previously reported in an Italian population with FM,[31] was found in four families, one of them of Italian ancestry, suggesting a possible founder effect[32]; IVS2-105A>G and p.M53I (c.159G>C), previously reported in melanoma-prone families from the United Kingdom, Australia, and the United States[7]; and mutation p.V43L (c.127G>C), affecting p14ARF, which has not previously been reported. In Uruguay we detected p.E88X (c.262G>T) in two families, which also was detected in two Spanish pedigrees. In Mexico we identified a mutation in the two probands of one family—p.I49T (c.146T>C)—which was previously reported in a case of FM by Hussussian et al.[33] and did not segregate with melanoma in that case. However, functional analysis showed impairment for this variant.[34]

We detected differences in *MC1R* variant distribution in our set of patients. Latin American patients with melanoma carry more *MC1R* variants. These genetic results correlate with the phenotypic data, where Latin American patients with melanoma have fairer skin and hair color. The prevalence of *MC1R* variants varies between populations.[35] In this study, specific variant frequencies differed between Latin American and Spanish patients with melanoma. Latin American patients with melanoma had an increased presence of p.R160W and p.R163Q. However, controls would be needed to assess the melanoma risk associated with carrying these variants in Latin America. p.R160W is associated with an increased risk for melanoma and red hair color.[10] By contrast, p.R163Q, which is not associated with pigmentation or tanning response, favors the development of chronic sun exposure melanomas in the Mediterranean population[22] and increases the risk for melanoma in areas with high ultraviolet radiation.[36] These reports suggest that a possible interaction between p.R163Q and a high ultraviolet radiation dose could favor melanoma development. Most Latin American countries receive a huge amount of ultraviolet radiation compared with northern latitudes; this could explain the increased frequency of SMP and FM with the p.R163Q variant in Latin America, although its frequency in a control Latin American population is unknown.

To date, genetic testing in patients at high risk for melanoma is restricted to *CDKN2A* and *CDK4*. More studies of patients wild type for these genes should be conducted to assess the role of other melanoma-susceptibility genes such as *MITF*, *BAP1*, *TERT*, *POT1*, *ACD*, and *TERF2IP*[8] for their possible incorporation in melanoma genetic counseling. In this study we demonstrated that *CDKN2A* germline mutation frequency in melanoma-prone families with at least two melanoma cases is greater in Latin America than Spain (23.9 vs. 14.1%, respectively). Inclusion criteria for genetic testing of melanoma in Spain follow the rule of two.[12] Based on the results of this

study, the inclusion criteria for genetic counseling for patients with melanoma in Latin America should also follow this rule because it allows the detection of *CDKN2A* mutations in a significant number of patients, except for southern Brazil, where the rule of three should be used. Genetic testing allows us to identify mutation carriers in families with a high risk of developing the disease. Carriers can be included in specific follow-up programs that allow the detection of melanomas at early stages, which improves the disease prognosis.[3,37,38] Digital follow-up with specific dermatologic techniques, including total-body photography and digital dermoscopy, allow early detection of melanomas with a low rate of excision.[38] Early melanomas in patients carrying *MC1R* variants may be difficult to diagnose definitively using dermoscopy, and an integrated approach including clinical history and dermoscopic data should be used when evaluating them.[39] Thus, *MC1R* sequencing could also help to choose the best screening methods. The experience of genetic counseling in Spain over 10 years shows that melanomas can be diagnosed at any time, so the follow-up of individuals at high risk for melanoma should be maintained over time.[12]

In conclusion, Latin American patients with melanoma and at high risk for melanoma had fair skin and European origin. The mutations found also had been detected in Spanish, European, or North American populations, suggesting that they could have a single origin and that there could be a founder effect. Finally, inclusion criteria for genetic counseling in Latin American patients with melanoma should follow the rule of two: two primary melanomas in an individual or families with at least one invasive melanoma and one or more other diagnoses of melanoma or pancreatic cancer in first- or second-degree relatives.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

The main funding for the study was provided by GenoMEL (contract LSHC-CT-2006–018702) and by the National Cancer Institute of the US National Institutes of Health (CA83115). The research at the Melanoma Unit in Barcelona is partially funded by grants 03/0019, 05/0302, 06/0265, 09/1393, and 12/00840 from Fondo de Investigaciones Sanitarias, Spain; by the *CIBER de Enfermedades Raras* of the Instituto de Salud Carlos III, Spain; by AGAUR 2014_SGR_603 of the Catalan Government, Spain; and by the European Commission under the 6th Framework Programme. M.P. is the recipient of a PhD Fellowship (PFIS) from Instituto de Salud Carlos III, Spain. F.C. was partially funded by a scholarship (152256/158706) from Consejo Nacional de Ciencia y Tecnología (CONACYT), México. The research at São Paulo, Brazil, was funded by Fundação para o Amparo da Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brazil (2007/04313-2). The research at Porto Alegre city, Brazil, was funded by the Brazilian Post-Graduation Agency Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The research in Uruguay was funded by Comisión Honoraria de Lucha Contra el Càncer and Fundación Manuel Pérez, Montevideo, Uruguay. The authors give special thanks to the coordinator of the RO1 grant, David Elder, and those of the FP6 grant, Julia Newton-Bishop and Nelleke Gruis, for their support in the development of the project. The authors also thank their patients and their families, who are the main reason for our studies; the nurses from the Melanoma Unit of Hospital Clínic of Barcelona; Daniel Gabriel, Pablo Iglesias, and Maria E. Moliner for helping to collect patient data; Amanda de Nobrega from São Paulo; Thomas Ruzicka, Carola Berking, and the Department of Dermatology and Allergology of Ludwig Maximilian University, Munich, Germany, for support in performing p.A148T analyses in Brazilian cases from HCPA and Carolina Ribas do Nascimento for performing *MC1R* tests in HCPA; Lídice Dufrechou from Uruguay and Helena Kruyer for helping with English editing and correction of the manuscript. In addition, the authors thank Victoria Godinez Puig from Mexico for helping to collect patient samples and data, as well as the Biobanco del Instituto Valenciano de Oncología and the A.C. Camargo Biobank.

Statement on prior presentation: part of the results of this study were presented as an Oral Communication in the GenoMEL/BioGenoMEL annual meeting 2014, Valencia, Spain, 7–9 May 2014.

Role of the sponsors: the sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; or in the preparation, review, or approval of the manuscript.

DISCLOSURE

The authors declare no conflict of interest.

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