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Original Investigation

Prevalence of *MITF* p.E318K in Patients With Melanoma Independent of the Presence of *CDKN2A* Causative Mutations

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IMPORTANCE The main high-penetrance melanoma susceptibility gene is *CDKN2A*, encoding p16INK4A and p14ARF. The gene *MITF* variant p.E318K also predisposes to melanoma and renal cell carcinoma. To date, the prevalence of *MITF* p.E318K and its clinical and phenotypical implications has not been previously assessed in a single cohort of Spanish patients with melanoma or in p16INK4A mutation carriers.

OBJECTIVES To evaluate the prevalence of *MITF* p.E318K in Spanish patients with melanoma and assess the association with clinical and phenotypic features.

DESIGN, SETTING, AND PARTICIPANTS A hospital-based, case-control study was conducted at the Melanoma Unit of Hospital Clinic of Barcelona, with *MITF* p.E318K genotyped in all patients using TaqMan probes. We included 531 patients: 271 patients with multiple primary melanoma (MPM) without mutations affecting p16INK4A (wild-type p16INK4A); 191 probands from melanoma-prone families with a single melanoma diagnosis and without mutations affecting p16INK4A, and 69 probands from different families carrying *CDKN2A* mutations affecting p16INK4A. A population-based series of 499 age- and sex-matched cancer-free individuals from the Spanish National Bank of DNA were included as controls. Patients were recruited between January 1, 1992, and June 30, 2014; data analysis was conducted from September 1 to November 30, 2014.

MAIN OUTCOMES AND MEASURES The genetic results of the *MITF* p.E318K variant were correlated with clinical and phenotypic features.

RESULTS Among the 531 patients, the prevalence of the *MITF* p.E318K variant was calculated among the different subsets of patients included and was 1.9% (9 of 462) in all melanoma patients with wild-type p16INK4A, 2.6% (7 of 271) in those with MPM, and 2.9% (2 of 69) in the probands of families with p16INK4A mutations. With results reported as odds ratio (95% CI), the *MITF* p.E318K was associated with an increased melanoma risk (3.3 [1.43-7.43]; $P < .01$), especially in MPM (4.5 [1.83-11.01]; $P < .01$) and high nevi count (>200 nevi) (8.4 [2.14-33.19]; $P < .01$). Two fast-growing melanomas were detected among 2 *MITF* p.E318K carriers during dermatologic digital follow-up.

CONCLUSIONS AND RELEVANCE In addition to melanoma risk, *MITF* p.E318K is associated with a high nevi count and could play a role in fast-growing melanomas. Testing for *MITF* p.E318K should not exclude patients with known mutations in p16INK4A. Strict dermatologic surveillance, periodic self-examination, and renal cell carcinoma surveillance should be encouraged in this context.

Editorial

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Approximately 8% to 12% of melanoma cases occur in a familial context.[1] The main high-penetrance gene implicated in melanoma susceptibility is *CDKN2A* (OMIM code: 600160; GenBank accession number: NM_000077.4 [p16INK4A] and NM_058195.3 [p14ARF]). The gene encodes 2 tumor suppressor proteins: p16INK4A, which promotes cell-cycle arrest and plays a role in senescence, and p14ARF, which acts through p53-regulating apoptosis.[2,3] Germline *CDKN2A* mutations are found in 20% to 40% of melanoma-prone families[4] and in 8% to 16% of patients with multiple primary melanomas without other cases in the family.[5] Patients with melanoma carrying *CDKN2A* mutations have a younger age of onset and a higher number of primary melanomas, and the presence of *CDKN2A* mutations is associated with dysplastic nevi.[4-7]

The *MC1R* gene (OMIM: 155555; GenBank: NM_002386) controls the pigmentation process and is a moderate-risk gene for melanoma susceptibility.[8,9] Loss of function variants in *MC1R* impairs the ability to activate the pigmentation pathway resulting in the red-hair color (RHC) phenotype. The RHC phenotype is characterized by fair pigmentation (fair skin, red hair, and freckles) and by sun sensitivity (poor tanning response and solar lentigines).[10] These variants increase the risk of melanoma with an odds ratio (OR) between 1.5 and 4.1.[8,10,11] The association between *MC1R* variants and melanoma is stronger in individuals with dark skin or few nevi.[12,13] Therefore, there might be a modest benefit to measure *MC1R* genotype for melanoma risk prediction, in addition to clinically measured pigmentation characteristics and nevi count.[12] A rare functional variant in *MITF*, p.E318K (rs149617956), was identified in 2 independent studies.[14,15] This variant may be considered as a moderate-risk allele in melanoma.[14-17] The master regulator gene of melanocyte development and differentiation is *MITF*, and it is also associated with melanoma development and progression.[18] The presence of *MITF* p.E318K predisposes to both familial and sporadic melanoma susceptibility, and/or renal cell carcinoma (RCC), and/or pancreatic cancer.[14-16] *MITF* p.E318K occurs at a conserved small ubiquitinlike modifier position, and this variant decreases the amount of small ubiquitinlike modifier–modified MITF forms.[14] The small ubiquitinlike modifier of MITF represses its transcriptional activity; therefore, p.E318K increases MITF transcriptional activity and may result in the upregulation of distinct sets of genes. Furthermore, this variant promotes invasive and tumorigenic behaviors in melanoma and RCC cells and might favor a phenotypic switch of melanoma cells toward a tumor-initiating cell phenotype.[14]

To our knowledge, the role of the *MITF* p.E318K has not been previously explored in Spanish patients with melanoma. The aim of this study was to evaluate the role of the *MITF* p.E318K variant in Spanish patients with melanoma and assess the association of this variant with clinical and phenotypic features.

Methods

Patients

A total of 531 patients at high risk of melanoma, recruited from January 1, 1992, to June 30, 2014, at the Melanoma Unit of Hospital Clinic of Barcelona, were included in the study. Patients were grouped into 3 different subsets. The first set was composed of 271 individuals (51%) with multiple primary melanoma (MPM) (212 sporadic MPM and 59 familial MPM) who did not carry mutations in *CDKN2A* affecting the p16INK4A protein (hereinafter referred to as wild-type p16INK4A). One patient from this set carried a *CDKN2A* mutation affecting p14ARF, and 75 patients were previously included in the Bertolotto et al[14] study. The second set consisted of 191 probands (36%) from melanoma-prone families, with at least 2 melanoma cases, with a single melanoma diagnosis (all wild-type p16INK4A). The third set contained 69 probands (13%) from families bearing *CDKN2A* mutations affecting p16INK4A, independent of the number of primary melanomas.

Clinical and phenotypic characteristics were collected for most of the 531 patients, including the number of primary melanomas (99%), age of onset (90%), melanoma subtype (76%), melanoma location (84%), Breslow thickness (74%), eye and hair color (75%), skin phototype (80%), and nevus count (72%). The familial history of pancreatic cancer was obtained from 80% of the patients, and personal history of other cancers was supplied by the patients carrying the variant.

The study was approved by the ethics committee of the Hospital Clinic of Barcelona, and patients provided written informed consent. Participants did not receive financial compensation.

A population-based series of 499 cancer-free individuals recruited at the Spanish National Bank of DNA were used as controls. The controls were sex and age matched with a group of consecutively recruited individuals with sporadic melanoma in the Melanoma Unit of Hospital Clinic of Barcelona from January 1998 to December 2013. The mean (SD) age in the control group was 52.2 (18.0) years. Overall, 269 of the 499 patients were women (53.9%) and 230 were men (46.1%).

MITF p.E318K Genotyping

The *MITF* variant p.E318K (rs149617956) was analyzed (Custom TaqMan SNP Genotyping Assays) according to manufacturer's recommendations in all of the patients and the control group. The process was carried out using polymerase chain reaction (7900HT Fast Real Time PCR System; Applied Biosystems) and SDS, version 2.4, software (Applied Biosystems).

Statistical Analysis

The prevalence of *MITF* p.E318K was assessed in all melanoma patients with wild-type p16INK4A and the *CDKN2A* mutation carrier set. In a French cancer-free and Italian control population, the frequency of carriers was 0.6% (14 of 2205).[14,16] The risk conferred by *MITF* p.E318K to melanoma development in the Spanish patients with wild-type p16INK4A melanoma was evaluated by comparing our group of patients with the Spanish and previously reported French and Italian controls together since there were no statistically significant differences between them. Clinical and phenotypic characteristics were analyzed regarding the presence of the p.E318K variant in patients with wild-type p16INK4A. Odds ratios and

Table 1. Melanoma Risk and Phenotypic Features According to the Presence of *MITF* p.E318K in Patients With Wild-Type p16INK4A

Characteristic	No. (%)		OR (95% CI)	*P* Value
	MITF p.E318K	WT		
Melanoma Risk[a]				
All patients	9 (1.9)	453 (98.1)	3.3 (1.43-7.43)	<.01
MPM	7 (2.6)	264 (97.4)	4.5 (1.83-11.01)	<.01
Phenotypic Features				
All MM patients				
>200 Nevi	4 (44.4)	28 (8.7)	8.4 (2.14-33.19)	<.01
Missing	0	130		
Fair skin	6 (66.7)	207 (57.3)	1.49 (0.37-6.04)	.74
Missing	0	92		
Non-blue eyes	8 (88.9)	264 (78.3)	2.21 (0.27-17.98)	.69
Missing	0	116		
Dark hair	6 (66.7)	232 (68.6)	0.90 (0.22-3.72)	>.99
Missing	0	115		
MPM				
>200 Nevi	4 (57.1)	19 (9.7)	12.4 (2.58-59.7)	<.01
Missing	0	68		
Fair skin	5 (71.4)	121 (58.5)	1.8 (0.34-9.37)	.70
Missing	0	57		
Non-blue eyes	6 (85.7)	140 (73.7)	2.1 (0.25-18.24)	.68
Missing	0	74		
Dark hair	5 (71.4)	126 (65.6)	1.3 (0.25-6.93)	>.99
Missing	0	72		
Familial history of pancreatic cancer				
Presence	1 (11.1)	15 (4.29)	2.9 (0.34-24.49)	.33
Missing	0	93		

Abbreviations: MM, malignant melanoma; MPM, multiple primary melanoma; OR, odds ratio; WT, wild-type.

[a] Data from 1659 French, 546 Italian, and 499 Spanish cancer-free controls were used. The *MITF* p.E318K frequency in the controls was 0.6% (16 of 2704).

95% CIs were calculated. The 2-sided Fisher exact test was used to look for statistical significance in proportion comparison. Age of onset was tested using an unpaired, 2-tailed *t* test. Breslow thickness was evaluated using the Mann-Whitney test. The results were considered statistically significant at $P < .05$. Statistical analyses were conducted using SPSS, version 17.0 (SPSS Inc). Data analysis was conducted from September 1 to November 30, 2014.

Results

CDKN2A gene, coding for the p16INK4A and p14ARF proteins, is a high-penetrance susceptibility in melanoma. Therefore, we calculated the prevalence of the *MITF* p.E318K variant in all 531 patients separated according to p16INK4A status (wild-type or mutated): 462 patients with wild-type p16INK4A and 69 patients with mutated p16INK4A. Among these, the prevalence of the *MITF* p.E318K variant was 1.9% (9 of 462) in all melanoma patients with wild-type p16INK4A, 2.6% (7 of 271) in those with MPM, and 2.9% (2 of 69) in the probands of families bearing a mutation in p16INK4A. All individuals with *MITF* p.E318K carried the variant in heterozygosis. The prevalence of the variant in a Spanish cancer-free population was 0.4% (2 of 499), with no statistically significant difference with French or Italian controls ($P = .54$).[14,16] With results reported as OR (95% CI), the *MITF* variant p.E318K increased the risk of developing melanoma in all melanoma patients with wild-type p16INK4A (3.3 [1.43-7.43]; $P < .01$) and in those with MPM (4.5 [1.83-11.01]; $P < .01$), using the Mediterranean controls (**Table 1**). When calculating the OR using, as a control population, the European non-Finnish population from the ExAC/Broad Institute exome database (http://exac.broadinstitute.org/, which gives an *MITF* p.E318K allele frequency of 0. 21% [140 *MITF* p.E318K alleles for 66732 total allele number]), the risk of developing melanoma in *MITF* p.E318K carriers was 4.7 (2.40-9.35; $P < .01$) and the risk of developing MPM was 6.3 (2.93-13.63; $P < .01$). We assessed the association between clinical and phenotypic features and the presence of p.E318K. The presence of the variant was associated with a very high nevi count (>200) in all patients with wild-type p16INK4A (8.4 [2.14-33.19]; $P < .01$) and in those with MPM (12.4 [2.58-59.7]; $P < .01$). We did not find any association with other phenotypical characteristics, family history of pancreatic cancer (Table 1), or clinicohistopathologic characteristics of tumors (**Table 2** and eTable 1 in the Supplement).

Table 3 reports the detailed clinical, phenotypic, and genetic characteristics of all patients with melanoma carrying *MITF* p.E318K. Patient M0881-01, with 3 previous melanomas, developed a fast-growing nodular melanoma that had not been present in a visit 2 months earlier that included total body photography. The patient detected a

Table 2. Clinicohistopathologic Features According to the Presence of *MITF* p.E318K in Patients With p16INK4A Wild-Type

Characteristic	*MITF* p.E318K	WT	OR (95% CI)	*P* Value
All Patients (N = 462)[a]				
Melanoma subtype, No. (%)[b]				
SSM	8 (100)	295 (86.3)	NA	.60
NM	1 (12.5)	35 (10.2)	1.25 (0.15-10.48)	.58
LMM	1 (12.5)	42 (12.3)	1.02 (0.12-8.50)	>.99
ALM	1 (12.5)	11 (3.2)	4.30 (0).49-38.02)	.25
Other	0	6 (1.8)	NA	>.99
Missing	1 (11.1)	111 (24.5)	NA	
Melanoma location, No. (%)				
Head or neck	2 (22.2)	54 (14.3)	1.71 (0.35-8.47)	.62
Trunk	5 (55.6)	198 (52.4)	1.13 (0.30-4.30)	>.99
Extremity	7 (77.8)	237 (62.7)	2.08 (0.43-10.16)	.50
Other	0	3 (0.8)	NA	>.99
Missing	0	75 (16.6)	NA	
MPM (n = 271)				
Melanoma subtype				
SSM	7 (100)	197 (92.1)	NA	.66
NM	1 (14.3)	22 (10.3)	1.5 (0.17-12.64)	.54
LMM	1 (14.3)	32 (15.0)	0.9 (0.11-8.14)	>.99
ALM	1 (14.3)	7 (3.3)	4.9 (0.52-46.62)	.23
Other	0	3 (1.4)	NA	>.99
Missing	0	51 (19.3)	NA	
Melanoma location				
Head or neck	1 (14.3)	38 (16.7)	0.8 (0.10-7.12)	>.99
Trunk	5 (71.4)	173 (75.9)	0.8 (0.15-4.21)	>.99
Extremity	6 (85.7)	129 (56.6)	4.6 (0.54-38.87)	.24
Other	0	2 (0.9)	NA	>.99
Missing	0	37 (14.0)	NA	
≥3 Primaries	2 (28.6)	51 (23.9)	1.2 (0.24-5.92)	.83
Breslow thickness, No. (median) [SD][c]				
All melanomas	12 (0.90) [1.54]	360 (0.76) [1.82]	NA	.43
First melanoma	5 (1.00) [2.26]	230 (0.89) [2.17]	NA	.33
Missing	1	119	NA	
Age at diagnosis of first melanoma, mean (SD), y [No.]	44.22 (16.60) [9]	46.83 (16.38) [406]	NA	.64
Missing, No.	0	47	NA	

Abbreviations: ALM, acral lentiginous melanoma; LMM, lentigo malignant melanoma; MPM, multiple primary melanoma; NA, not applicable; NM, nodular melanoma; SSM, superficial spread melanoma; WT, wild-type.

[a] There was a total of 462 patients. The number indicates the total number of primary melanomas diagnosed with the subtype or location indicated in each row, within each group of patients (*MITF* p.E318K carriers or patients with the WT *MITF*).

[b] Data on subtypes were determined based on the number of patients with at least 1 tumor of this subtype or location. Data on missing information refers to the total number of patients with missing information.

[c] In situ melanomas were not considered when calculating the Breslow median thickness (millimeters).

fast-growing hypopigmented lesion on the elbow that arose 3 weeks before an urgent evaluation at our unit (**Figure 1**). With dermoscopy, the lesion showed an unspecific pattern, with asymmetry in the distribution of colors and structures, the presence of blue-gray color, and milky-red areas with some vessels. In confocal microscopy, the lesion showed some bright, large, round cells in the upper epidermis around a central ulceration; in the dermoepidermal junction, papilla were not well demarked and not visible in some areas with dermal nests of noncohesive bright cells with large nuclei, highly suggestive of melanoma. The lesion was excised the same day with the final diagnosis of nodular melanoma with Breslow thickness of 1.3 mm, ulceration, 5 mitoses/mm^2, and epithelioid cells. Wide excision was performed and sentinel lymph node biopsy identified 3 negative sentinel lymph nodes.

Patient M1340-01, in follow-up for recurrent lentigo maligna melanoma, had also developed a fast-growing melanoma, in this case amelanotic, that had not been present in a visit 4 months earlier. Under dermoscopy, the lesion also showed an unspecific pattern with remnants of pigmentation, the presence of doted and linear irregular vessels, and short white streaks. The lesion was excised and the diagnosis was superficial-spreading melanoma in a vertical growth phase with a Breslow thickness of 1.65 mm, lack of ulceration, 3 mitoses/mm^2, and fusocellular morphology. Wide excision was performed, and sentinel lymph node biopsy identified 3 negative sentinel lymph nodes.

Patient M3879-01 belonged to a family with 2 cases of melanoma; thus, we assessed whether the other patient carried the variant. In this case, *MITF* p.E318K did not segregate with melanoma; however, the carrier was younger at the time of diag-

Table 3. Clinical, Phenotypic, and Genetic Features of the Spanish *MITF* p.E318K Carriers

Identification, Patient	No. of Melanomas	Sex/Age of Onset, y	Hair/Eye Color	Phototype[a]	Nevi Count	Other Tumors	*CDKN2A* Mutations		MC1R Missense Variants[b]
							p16INK4A	p14ARF	
M0109-01	NA	M/NA	NA/NA	NA	NA	NA	p.D84Y	p.R98L	R142H
M0881-01	4	M/40s	Brown/brown	II	>200	BCC	No	p.G32R	I155T
M1340-01	2	M/60s	Brown/blue	II	<50	BCC and RCC	No	No	R151C and V92M
M1545-01	2	F/40s	Blond/green	II	>200	BCC	No	No	V60L
M1569-01	4	M/20s	Blond/green	II	100-200	No	No	No	WT
M3824-01	2	F/70s	Brown/brown	II	<50	No	No	No	WT
M3879-01	2	F/30s	Brown/brown	III	>200	No	No	No	WT
M3879-03	0	F/NA	Black/brown	II	>200	No	No	No	V60L
M3879-09	0	F/NA	Brown/brown	II	100-200	No	No	No	WT
M4182-01	2	M/20s	Brown/brown	III	>200	No	No	No	R160W
M4619-01	1	M/30s	Blond/green	II	100-200	No	No	No	V92M
M4713-01	1	M/40s	Brown/brown	III	50-100	No	No	No	V92M
M4999-01	1	F/40s	Blond/green	II	>200	No	p.A127S	No	R163Q

Abbreviations: BCC, cutaneous basal cell carcinoma; NA, not available; RCC, renal cell carcinoma; WT, wild-type.

[a] The phototype is indicated using the Fitzpatrick Scale.

[b] All variants were detected in heterozygosis.

nosis (30s vs 70s). We also detected 2 healthy individuals carrying the variant in this family: M3879-03 and M3879-09 (phenotypic features of those 2 individuals are also recorded in Table 3). The nevi from carriers followed a reticular pattern and were dark brown (**Figure 2**).

Discussion

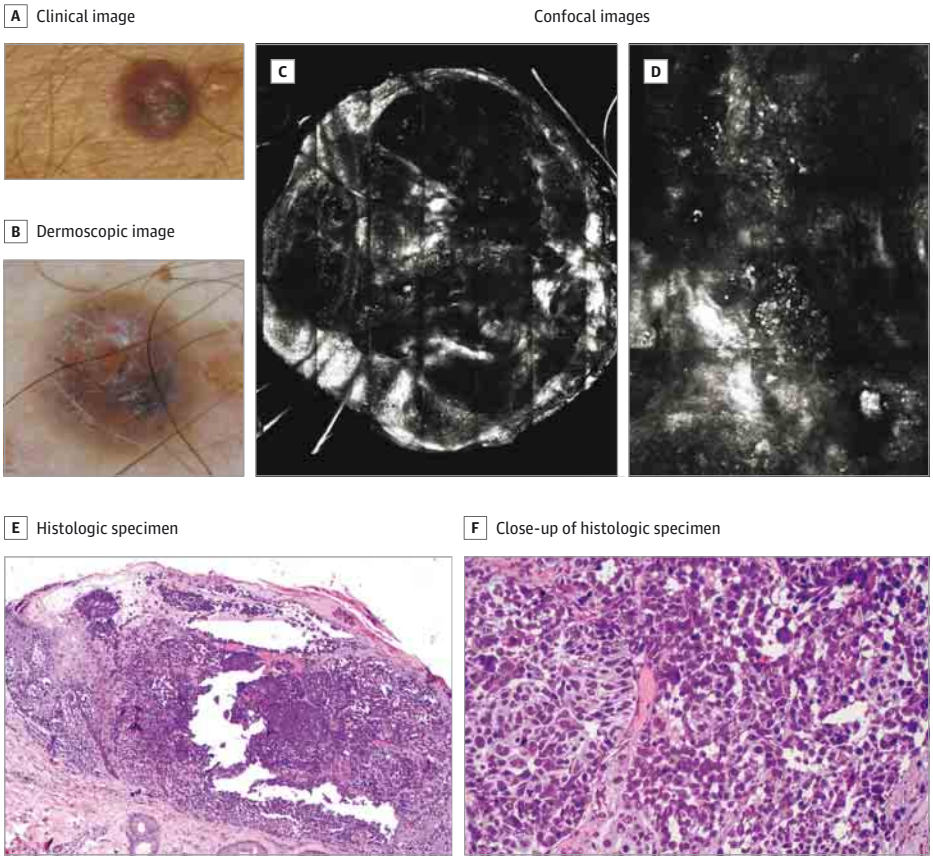
In this study, we analyzed the prevalence of *MITF* p.E318K in Spanish patients with melanoma. To our knowledge, this is the first study in which a set of individuals bearing mutations in *CDKN2A* affecting p16INK4A was also tested for *MITF* p.E318K. We detected a prevalence of 1.9% of the variant in all patients with wild-type p16INK4A, which was higher in the MPM subgroup (2.6%), and a similar prevalence was found in the set of patients with the p16INK4A mutation (2.9%). Previous studies[14-17,19] reported that *MITF* p.E318K increases the risk of developing melanoma (eTable 2 in the Supplement). We also detected this association in our set of patients. In addition to reporting increased melanoma risk, Yokoyama and colleagues[15] stated that the presence of this variant was associated with a high nevi count in an Australian and UK population. We also found that *MITF* p.E318K is associated with a very high nevi count (>200 nevi) in a Mediterranean population. These findings suggest that *MITF* may be involved in nevogenesis. Twin studies[20-22]have revealed evidence that the nevi count is genetically determined, with an additive genetic variance of 36% to 84%, increasing with age. The nevi count is a polygenic trait determined by multiple alleles.[23-27] The present study and the results in Australian and UK populations indicate that *MITF* p.E318K should be included in the set of known genes involved in this phenotypical trait.

To our knowledge, only one study[19] has described the phenotypical features and dermoscopic pattern of nevi from *MITF* p.E318K carriers in Australian patients. The investigators observed that these carriers had pink or light brown nevi, suggesting that *MITF* p.E318K could modulate nevi pigmentation. In contrast, in our study, the nevi were dark brown, indicating that other genes may be involved in this feature. The *MC1R* gene may modulate the pigmentation of the nevi since melanomas from carriers of RHC variants are less pigmented.[28] Otherwise, in the Australian study[19] and ours, the dermoscopic pattern of nevi present in *MITF* p.E318K carriers was predominantly reticular. These findings are suggestive of photoinduced nevogenesis.[29]

Sturm and colleagues[19] noticed a high incidence of amelanotic melanoma within *MITF* p.E318K carriers. One of our patients (M1340-01) also developed this type of melanoma. The patient carried one *MC1R* RHC variant: p.R151C. However, although our findings support the hypothesis that a genetic interaction between *MC1R* RHC and *MITF* p.E318K could increase the risk of developing amelanotic melanomas, it has been reported[17] that the interaction of *MITF* and *MC1R* variants is not associated with melanoma pigmentation.

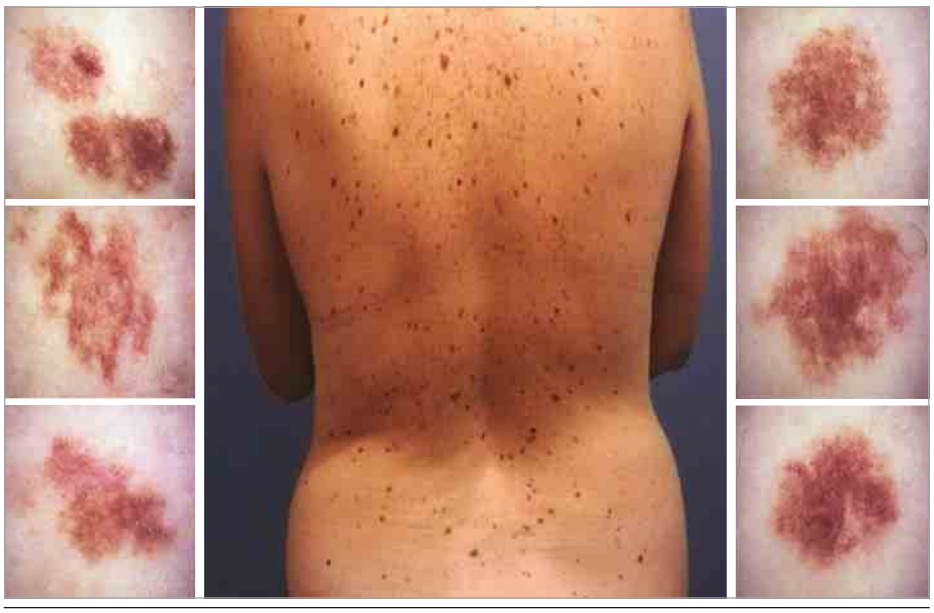
Ghiorzo and colleagues[16] found an association between *MITF* p.E318K and the presence of nodular melanomas. We did not detect a significant association with any clinicohistopathologic features, probably because the sample size lacked the power to detect these possible associations. However, we noted 2 fast-growing melanomas in 2 *MITF* p.E318K carriers who were receiving dermatologic surveillance owing to a previous melanoma diagnosis. Dermatologic digital follow-up has been demonstrated[30,31] to be relevant for detecting melanomas at early stages with a low rate of excisions in patients at high risk to develop melanoma. During 10 years of dermatologic surveillance of patients at high-risk of melanoma in our melanoma unit (from January 1, 1999, to December 31, 2008), 98 new melanomas were diagnosed in these patients; 54% were in situ melanoma and 46% were invasive melanoma. Among the invasive melanomas diagnosed, none was more than 1-mm Breslow thickness and no melanomas behaved as fast-

Figure 1. Melanoma



A fast-growing melanoma developed within 3 weeks and was the fourth to occur in patient M0881-01. Clinical picture of a 4-mm-diameter nodular lesion located on the elbow (A); dermoscopic image of the lesion showing hypopigmentation, asymmetry, unspecific pattern, atypical vessels, and blue-whitish veil (B). Under confocal microscopy, the lesion shows an ulcerated central area (C) with atypical nests in upper dermis with bright roundish nucleated cells in noncohesive nests (D). Histopathologic examination shows an ulcerated nodular melanoma (hematoxylin-eosin, original magnification ×2) (E) and nests of atypical cells and presence of mitosis (hematoxylin-eosin, original magnification ×10) (F).

Figure 2. Nevi



The back of patient M3879-01 with 2 previous melanomas and more than 200 nevi. Six dermoscopic images show the predominant pattern, reticulated dark brown.

growing melanomas.[31,32] Until now in our melanoma unit, the only 2 fast-growing melanomas identified by dermatologic digital follow-up in individuals at high risk of melanoma were in *MITF* p.E318K carriers. Fast-growing melanomas are defined by having a growth rate of greater than 0.4 mm per month; in general, the melanoma growth rate is approximately 0.1 mm per month, and slow-growing melanomas usually have a growth rate of 0.01 mm per month.[33] Furthermore, a high growth rate is

associated with a worse prognosis in melanoma; thus, strategies for early detection of fast-growing melanomas are necessary.[34] Although further studies should address the role of *MITF* p.E318K in fast-growing melanoma, the carriers of *MITF* p.E318K should be encouraged to perform monthly total-body self-examination of the skin and receive fast-track, urgent dermatologic visits if any new lesion appears.

Genetic counseling is increasingly being offered to patients with sporadic MPM or familial melanoma and/or to their healthy relatives.[35] The genetic counseling in melanoma is focused on the screening of high-penetrance genes such as *CDKN2A*. Although *MITF* p.E318K is a moderate melanoma risk allele, it also increases the risk of developing RCC and pancreatic cancer. In our set of carriers, we observed that 42.9% (3 of 7) of the patients carrying *MITF* p.E318K developed cutaneous basal cell carcinoma, which is similar to previous data reported in wild-type *CDKN2A* MPM.[36] We did not detect any association with pancreatic cancer, probably owing to the small number of carriers in the study. Reinforcing their predisposition to develop RCC, 14.3% (1 of 7) carriers had developed this kind of tumor. Thus, if genetic counseling included *MITF* p.E318K genetic testing, individuals carrying the *MITF* p.E318K variant could benefit from being included both in melanoma and RCC prevention/surveillance programs. Furthermore, the detection of *MITF* p.E318K may identify patients at risk of developing fast-growing melanomas. We have observed a similar prevalence of *MITF* p.E318K in cases with germline *CDKN2A* mutations as in patients with wild-type. However, further studies should be performed to assess the role of *MITF* p.E318K as a possible modulator of the effect of *CDKN2A* mutations. This result suggests that individuals with a mutation in *CDKN2A* might also be included in *MITF* p.E318K screening as the identification of this variant allows for better characterization of the risk in the family and to adapt the cancer surveillance programs accordingly.

Patients with *MITF* p.E318K should be encouraged to follow melanoma prevention programs, which include sun protection strategies, monthly self-examination of the skin, and dermatologic surveillance. Because *MITF* p.E318K has been associated with RCC,[14] the use of renal ultrasonography as a safe and low-cost screening technique to detect the presence of kidney tumors should be considered. Future studies should explore the cost-efficacy and acceptance of this screening technique in *MITF* p.E318K carriers. It would be important to recommend that carriers avoid smoking and control their weight since smoking and obesity are important risk factors for both RCC and pancreatic cancer.[37,38] Moreover, detection of *MITF* p.E318K in a patient leads to the possibility of extending genetic testing to other relatives, and positive cases should be encouraged to follow the same preventive measures.

Conclusions

Based on the results of this study, *MITF* (and *MC1R*) should be added to *CDKN2A/CDK4* genetic testing based on published international recommendations for countries with low and high sun exposure.[39] Genotyping for *MITF* (and *MC1R*) could be added to predictive testing for all relatives in *CDKN2A*-positive families.

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