Received: 1 June 2023 | Accepted: 11 August 2023

DOI: 10.1111/jdv.19461

JEADV JOURNAL OF THE EUROPEAN ACADEMY OF DERMATOLOGY & VENEREOLOGY

ORIGINAL ARTICLE

High- and intermediate-risk susceptibility variants in melanoma families from the Mediterranean area: A multicentre cohort from the MelaNostrum Consortium

C. Pellegrini[1] | L. Cardelli[1] | P. Ghiorzo[2,3] | L. Pastorino[2,3] | M. Potrony[4,5,6] | Z. García-Casado[7] | L. Elefanti[8] | I. Stefanaki[9] | M. Mastrangelo[1] | S. Necozione[10] | P. Aguilera[5,6,11] | A. Rodríguez-Hernández[12] | L. Di Nardo[13,14] | T. Rocco[1,15] | L. Del Regno[13,14] | C. Badenas[4,5,6] | C. Carrera[5,6,11] | J. Malvehy[5,6,11] | C. Requena[12] | J. Bañuls[16] | A. J. Stratigos[9] | K. Peris[13,14] | C. Menin[8] | D. Calista[17] | E. Nagore[12] | S. Puig[5,6,11] | M. T. Landi[18] | MC. Fargnoli[1,15]

[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

[2]IRCCS Ospedale Policlinico San Martino, Genetica dei Tumori rari, Genoa, Italy

[3]Department of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy

[4]Department of Biochemistry and Molecular Genetics, Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, Spain

[5]Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain

[6]Biomedical Research Networking Center on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain

[7]Laboratory of Molecular Biology, Instituto Valenciano de Oncología, València, Spain

[8]Immunology and Diagnostic Molecular Oncology Unit, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy

[9]1st Department of Dermatology-Venereology, Andreas Sygros Hospital, National and Kapodistrian University of Athens, Athens, Greece

[10]Epidemiology Unit, Department of Life, Health and Environmental Science, University of L'Aquila, L'Aquila, Italy

[11]Department of Dermatology, Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, Spain

[12]Department of Dermatology, Instituto Valenciano de Oncología, València, Spain

[13]UOC Dermatologia, Dipartimento di Scienze Mediche e Chirurgiche, Fondazione Policlinico Universitario A. Gemelli – IRCCS, Rome, Italy

[14]Dermatologia, Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Rome, Italy

[15]Dermatology Unit, Ospedale San Salvatore, L'Aquila, Italy

[16]Department of Dermatology, Hospital General Universitario de Alicante, Alicante, Spain

[17]Department of Dermatology, Maurizio Bufalini Hospital, Cesena, Italy

[18]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Correspondence
MC. Fargnoli, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Via Vetoio, Coppito II, 67100, L'Aquila, Italy.
Email: mariaconcetta.fargnoli@univaq.it

Abstract

Background: Most of large epidemiological studies on melanoma susceptibility have been conducted on fair skinned individuals (US, Australia and Northern Europe), while Southern European populations, characterized by high UV exposure and dark-skinned individuals, are underrepresented.

C. Pellegrini and L. Cardelli contributed equally as first authors.

E. Nagore, S. Puig, M. T. Landi and MC. Fargnoli contributed equally as last authors.

Linked article: S. Ribero et al. J Eur Acad Dermatol Venereol 2023;37:2405–2406. https://doi.org/10.1111/jdv.19529.

Funding information
Fondo de Investigaciones Sanitarias (Spain), Grant/Award Number: PI18/00419, PI15/00956 and PI15/00716; CIBER de Enfermedades Raras of the Instituto de Salud Carlos III; ISCIII-Subdireccion General de Evaluacion and European Regional Development Fund (ERDF); AGAUR, Grant/Award Number: 2017_SGR_1134; CERCA Programme by Generalitat de Catalunya; European Commission under the 6th Framework Programme, Grant/Award Number: LSHC-CT-2006-018702; European Commission under the 7th Framework Programme; Diagnoptics; European Commission under the HORIZON2020 Framework Programme; iTobos and Qualitop; The National Cancer Institute (NCI) of the US National Institute of Health (NIH), Grant/Award Number: CA83115; Fundació La Marató de TV3, Grant/Award Number: 201331-30; Fundación Científica de la Asociación Española Contra el Cáncer, Grant/Award Number: GCB15152978SOEN; Italian Ministry of Health, Grant/Award Number: RF-2016-02362288; IRCCS Ospedale Policlinico San Martino

Objectives: We report a comprehensive pooled analysis of established high- and intermediate-penetrance genetic variants and clinical characteristics of Mediterranean melanoma families from the MelaNostrum Consortium.
Methods: Pooled epidemiological, clinical and genetic (*CDKN2A*, *CDK4*, *ACD*, *BAP1*, *POT1*, *TERT*, and *TERF2IP* and MC1R genes) retrospective data of melanoma families, collected within the MelaNostrum Consortium in Greece, Italy and Spain, were analysed. Univariate methods and multivariate logistic regression models were used to evaluate the association of variants with characteristics of families and of affected and unaffected family members. Subgroup analysis was performed for each country.
Results: We included 839 families (1365 affected members and 2123 unaffected individuals). Pathogenic/likely pathogenic *CDKN2A* variants were identified in 13.8% of families. The strongest predictors of melanoma were ≥2 multiple primary melanoma cases (OR 8.1; 95% CI 3.3–19.7), >3 affected members (OR 2.6; 95% CI 1.3–5.2) and occurrence of pancreatic cancer (OR 4.8; 95% CI 2.4–9.4) in the family (AUC 0.76, 95% CI 0.71–0.82). We observed low frequency variants in *POT1* (3.8%), *TERF2IP* (2.5%), *ACD* (0.8%) and *BAP1* (0.3%). *MC1R* common variants (≥2 variants and ≥2 RHC variants) were associated with melanoma risk (OR 1.4; 95% CI 1.0–2.0 and OR 4.3; 95% CI 1.2–14.6, respectively).
Conclusions: Variants in known high-penetrance genes explain nearly 20% of melanoma familial aggregation in Mediterranean areas. *CDKN2A* melanoma predictors were identified with potential clinical relevance for cancer risk assessment.

INTRODUCTION

Melanoma is one of the most aggressive cancers, with an incidence that varies widely among white-skinned populations and has increased significantly in recent years.[1,2]

Major risk factors include ultraviolet (UV) radiation, fair phenotype and atypical melanocytic nevi. Inherited genetic variants are a key component of melanoma risk, with an estimated heritability of 55%–58% in twin studies.[3,4]

Approximately 5%–12% of melanomas are diagnosed in familial contexts.[5,6] Individuals with one first-degree relative with melanoma have approximately twofold (range 1.7–13.6) higher risk of developing the disease than the general population and the risk increases with the number of affected relatives.[7] Within the family, melanoma patients often have an early diagnosis, multiple primary melanomas (MPM), and may develop other malignancies.[8–10]

Genetic variants in high-penetrance genes account for 20%–45% of familial melanomas.[6,11] *CDKN2A* is the major melanoma susceptibility gene, with germline variants identified in 10%–40% of familial cases.[12–15] Clinically, *CDKN2A* variants have been associated with a high number of affected family members, MPM and pancreatic cancer although the effects vary across populations.[12,15,16–23]

Pathogenic variants in other established high-risk genes contribute to less than 10% of heritability.[5,6,24] *CDK4* variants, occurring in exon 2, have been associated with early diagnosis.[25] Variants in telomere-linked genes (*TERT*, *POT1*, *ACD*, *TERF2IP)* have been described in families with a high density of additional cancers.[24,26–29] Germline *BAP1* variants have been identified in melanoma families with aggregation of uveal melanoma, mesothelioma, renal cell carcinoma and atypical intradermal melanocytic tumours.[30,31] Other recently suggested susceptibility genes, such as *GOLM1*, *EBF3*, *POLE* and *NEK11*, need to be validated.[11]

Missing melanoma heritability has been related to inheritance of multiple low- to intermediate-risk alleles and/or shared environmental exposure. The major known low- to intermediate-risk genes are involved in melanogenesis (*MC1R*, *MITF*, *OCA2*, *SLC45A2*, *TYR*, *TYRP1*), DNA synthesis, immunity pathways, cell–cell junction and transcriptional regulation (*ATM*, *HLA locus*, *CDH1*, *FOXD3*, *SOX10*).[5,32] Variants in *MC1R* are the most common and established genetic trait that predisposes to cutaneous melanoma.[33] The strongest impact on susceptibility is attributed to *MC1R* RHC (red hair colour) alleles and to the presence of multiple variants.[34] Notably, *MC1R* polymorphisms increase the penetrance of *CDKN2A* mutations in melanoma-prone families.[34]

Since the 1990s, most studies on melanoma susceptibility have been conducted in populations of Celtic origin (United States, Australia and Northern Europe)[12,16–18,20,22,35–37] while Southern European populations, that are characterized by high UV exposure and a high proportion of dark-skinned individuals, have been underrepresented. Melanoma susceptibility has been investigated in families from individual countries of the Mediterranean area; however, a comprehensive pooled evaluation is lacking.

We report a pooled analysis of clinical characteristics and genetic variants of established predisposition genes in a large cohort of Southern European families collected across the MelaNostrum Consortium,[1] to identify distinctive features of hereditary melanoma in the Mediterranean area.

MATERIALS AND METHODS

Study population

A total of 881 melanoma families were retrospectically collected from eight centres in Greece, Italy and Southern Spain participating in the MelaNostrum Consortium (https://dceg.cancer.gov/research/cancer-types/melanoma/melanostrum).[1] Inclusion criteria were the occurrence of histopathologically confirmed melanoma in at least two first or second degree relatives and availability of *CDKN2A* genetic data for at least one affected member in the family. Seven centres participated with families not selected for *CDKN2A* status, while one centre (Genoa) provided *CDKN2A*-negative families (Table S1).

We collected information on phenotypic and clinical characteristics, and genetic data for families, affected and unaffected members from each centre (Supplementary Methods in Appendix S1). All data were harmonized to create a uniform multicentric pooled database, subdividing information on families and on individuals (patients and unaffected relatives). Details of data collection are reported in the Supplementary Methods in Appendix S1.

Informed consent was obtained from participants at each centre under local Institutional Review Board–approved protocols, as reported in the Appendix S1.

Genetic data

Germline screening of *CDKN2A*, *CDK4* and *MC1R* was performed via target sequencing at each recruiting centre. Genetic data for *POT1*, *BAP1*, *TERF2IP*, *ACD* and *TERT* in families *CDKN2A/CDK4*-negative were obtained by whole exome sequencing at NCI, USA (Supplementary Methods in Appendix S1).

Variants were annotated using different interpretation tools and databases (Varsome and ClinVar) and further classified as class-1 (benign), class-2 (likely benign), class-3 (variants of uncertain significance, VUS), class-4 (likely pathogenic) and class-5 (pathogenic) according to ACMG guidelines.[38]

MC1R variants were categorized as RHC variants (D84E, R142H, R151C, R160W, D294H, I155T) and non-RHC (NRHC) variants (all other missense changes).[39]

Statistical analysis

For high-penetrance genes, we performed two analyses, one for families reported in the main text and the other for individuals (affected and unaffected family members) described in the Supplementary Results in Appendix S1. For *MC1R*-intermediate gene, we analysed individuals and reported the results in the main test.

The *CDKN2A* mutational status was classified based on pathogenicity as wild-type/classes 1–2, class 3 and classes 4–5. For other high-risk genes, patients were categorized as wild-type vs presence of any variant. For *MC1R*, individuals were further divided into 'RHC' patients, with at least one RHC variant, and 'NRHC' patients, carrying all other variants; synonymous polymorphisms were excluded.

For each gene, we analysed the frequency of variants overall and for each country. The 78 families from Genoa, which were negative for *CDKN2A* mutations, were excluded from the calculation of the overall and Italian frequency of *CDKN2A* variants. Wilcoxon's test and chi-square or Fisher's exact tests were used for univariate analysis. We built a logistic regression model to assess the association between CDKN2A genetic status and specific predictors, as number of affected members (2; 3 and >3), number of MPM patients (0; 1; ≥2), grade of relatedness (first; second), presence of pancreatic cancer (yes; no), presence of other cancers (yes; no), age at diagnosis ≤50 years (yes; no) and country), used as covariates. We calculated the odds ratio (OR) with the 95% confidence interval (CI). The ability of the model to predict familial occurrence of class 4–5 variants was estimated through the ROC and calculation of the area under the curve (AUC) derived from the set of predictive odds, in the entire cohort and for each country.

We also compared the association between *MC1R* variants and melanoma status (affected members vs. unaffected relatives) to assess the risk of melanoma in *MC1R* carriers by a logistic regression model including only *MC1R* genotyping, after adjustment for sex, country and *CDKN2A* status.

Missing data were not present in clinical variables referring to families. Regarding the individuals (patients and unaffected family members), variables on phenotype, such as hair and eye colour, nevi number and skin type, showed missing data. These categorical variables were used only for univariate analysis and cases with missing data were excluded from the Chi-square test calculation.

Statistical analysis was performed with SPSS (IBM) 25.0 (SPSS Inc.). Results were considered statistically significant with a p-value < 0.05.

RESULTS

Family cohort

Overall, 881 melanoma families were collected from the database of the MelaNostrum Consortium. Of these, we included 839 families that had first or second degree affected relatives and available *CDKN2A* data. Clinical and genetic data of the 839 melanoma families were evaluated, including 1365 patients and 2123 unaffected relatives, for a total of 3488 individuals (Figure 1a). Of the 839 families, 520 (62.0%) were from Spain, 305 (36.3%) from Italy and 14 (1.7%) from Greece. The number of melanoma patients per family ranged from 2 to 10, with a median of two cases. Demographic and clinical characteristics of families and individuals are described in Table 1 and Supplementary Results in Appendix S1.

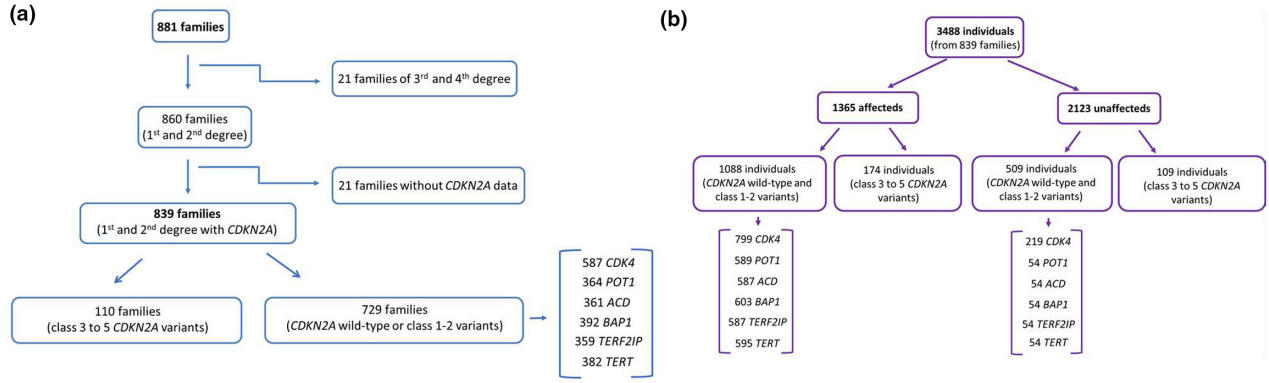

FIGURE 1 Flow chart of families and individuals included in the study. *Left panel*: of the 881 recruited families, we included 839 families with first or second degree affected relatives and available *CDKN2A* data. Of the 839 families, we collected genetic data of other high-risk genes for 734 families that were *CDKN2A* wild-type or carried *CDKN2A* class 1 or class 2 variants. Of these, the number of families with available data for each high-risk gene other than *CDKN2A* is reported into brackets. *Right panel*: we included 3488 individuals from 839 families: 1365 affected and 2123 unaffected members. The number of individuals for whom we had data on other high-risk genes is shown into brackets. Families from Genoa were excluded when calculating the frequency rate of *CDKN2A* variants. Greek cases were not included in the evaluation of other high-risk genes besides *CDKN2A*, since they were not centrally analysed at the Division of Cancer Epidemiology and Genetics, National Cancer Institute. The definition of familial melanoma is according to Gandini S, et al. *Eur J Cancer*. 2005;41(14):2040–2059.

CDKN2A variants in families

We identified 42 *CDKN2A* variants: 21.4% were mapped to exon 1α, 57.1% to exon 2 and only two mutations to exon 1β. In detail, 64.3% were missense, 16.7% non-coding, 7.1% deletions, 7.1% nonsense and 4.8% insertions (Table 2).

Overall, 69.0% of variants were classes 4–5 and were detected in 13.8% of families. Stratifying by country, 42.9% of Greek families, 14.4% of Spanish and 10.6% of Italian carried class 4–5 mutations. Class 3 variants were 11.9% of the total and were detected in 0.7% of families, while classes 1–2 in 1.8% of the families. The remaining 83.7% of families were *CDKN2A* wild type.

The G101W variant was the prevalent variant, identified in 34 (27.4%) families; of these, 17.6% and 82.4% were Italian and Spanish families, respectively. Greek families were not carriers of this variant. The second most common variant was V59G detected in 10 (8.1%) families, mainly Spanish and non-Greek. The R24P variant, identified in 9 (7.3%) families, was present in Greece (6/9, 66.7%) and Italy (3/9, 33.3%) but not in Spain.

The logistic model identified the presence of MPM cases (OR 4.4, 95% CI 2.8–6.9, $p<0.001$), the occurrence of pancreatic carcinoma (OR 4.8, 95% CI 2.4–9.4, $p<0.001$) and a large number of affected members (OR 2.0, 95% CI 1.3–3.2, $p=0.003$) as independent predictors of *CDKN2A* class 4–5 variant carriers. The OR was 8.1 (95% CI 3.3–19.7, $p<0.001$) for families with ≥2 MPM patients and 2.6 (95% CI 1.3–5.2, $p=0.009$) for those with >3 affected members (Table 3). The AUC was 0.76 (95% CI 0.71–0.82, $p<0.001$), indicating a good ability to discriminate between families with and without *CDKN2A* mutation (Figure S1). After stratification by country, the estimated probability of carrying a class 4–5 variant showed a good level of prediction in Southern Spain (AUC 0.79, 95% CI 0.73–0.85, $p<0.001$) and in Italy (AUC 0.73, 95% CI 0.63–0.84, $p<0.001$; Figure S1), although with some differences (Table 3). The small numbers of Greek families precluded a separate evaluation of the probability.

Other high-risk genes in families

Genetic data of *POT1*, *BAP1*, *TERF1P*, *ACD* and *TERT* were analysed for families negative for *CDKN2A* class 3–5 variants. The list of variants and their predicted functions are reported in Table 4.

Overall, 9 *POT1* mutations were identified in 14 families (3.8%). The most frequent mutation was the S270N, detected in 35.7% of the mutated families, all from Italy. The presence of *POT1* variants was associated with the occurrence of MPM cases in the families ($p=0.03$) and with greater Breslow thickness (median value 1.05 mm, range 0.47–4.5) of melanomas than those in wild-type patients (median value 0.7 mm, range 0.1–28.0; $p=0.04$).

Variants in *TERF2IP* were identified in 2.5% of our families. Among the seven *TERF2IP* variants, the majority were missense, and only one was nonsense (Q354*). In *ACD*, we observed two missense variants, detected in 0.8% of families. *TERT* variants were identified in two probands from two families, carrying the I729V and G424S mutations respectively, while the pathogenic −57 T>G promoter variant was not present in our cohort.

Regarding *BAP1*, we detected the S455P mutation in two members of one family, both with MPM.

Of note, no *CDK4* variants were identified.

MC1R gene in affected and unaffected members

Mutational status of *MC1R* gene was available for 932 patients and 483 unaffected members. *MC1R* variants were present in 65.9% of affected and 60.4% of unaffected members (OR 1.3, 95% CI 1.0–1.6, $p=0.044$, univariate analysis). RHC variants

TABLE 1 Demographic and clinical characteristics of families, patients and unaffected relatives included in the study.

Families		***N*** **= 839 (%)[a]**
Country	Greece	14 (1.7)
	Italy	305 (36.3)
	Spain	520 (62.0)
Grade of relatedness	First degree	713 (85.0)
	Second degree	126 (15.0)
No. of affected members	2 members	629 (75.0)
	3 members	154 (18.3)
	>3 members	56 (6.7)
Number of MPM patients in families	Single	597 (71.2)
	Multiple	242 (28.8)
	1 MPM patient	212 (25.3)
	2 MPM patients	29 (3.4)
	3 MPM patients	1 (0.1)
Family history of cancer[b]	No	259 (30.9)
	Yes	560 (66.7)
	Breast	161 (28.7)
	Lung	122 (21.8)
	Colon	92 (16.4)
	Prostate	76 (13.6)
	Pancreas	51 (9.1)
	Kidney	26 (4.6)
	Sarcoma	11 (2.0)
	Other	197 (35.2)

Individuals		**Affected** ***N*** **= 1365 (%)[a]**	**Unaffected** ***N*** **= 2123 (%)[a]**
Sex	Female	803 (58.8)	1083 (51.0)
	Male	562 (41.2)	974 (45.9)
Age	Median Age (range)	45.5 (7–81)	-
	≤50 years	799 (60.3)	-
	≤40 years	508 (37.2)	-
Hair colour	Red	76 (5.5)	26 (1.2)
	Blond	240 (17.6)	88 (4.1)
	Brown	684 (50.1)	595 (28.0)
	Black	81 (6.0)	76 (3.6)
Eye colour	Light	657 (48.1)	227 (10.7)
	Medium/Dark	485 (35.5)	480 (22.6)
Skin type	I	73 (5.3)	32 (1.5)
	II	568 (41.6)	251 (11.8)
	III	413 (30.3)	311 (14.6)
	IV	97 (7.1)	125 (5.9)
	V	7 (0.5)	5 (0.2)
Number of melanocytic nevi on total body	<10	122 (9.0)	35 (1.6)
	10–50	332 (24.3)	124 (5.8)
	>50	423 (31.0)	120 (5.6)
Personal history of other cancers	No	1144 (83.8)	1952 (92.0)
	Yes	221 (16.2)	162 (7.6)
	Keratinocyte carcinoma	118 (53.4)	Na
	Breast	34 (15.4)	22 (13.6)
	Lung	14 (6.3)	20 (12.3)
	Prostate	10 (4.5)	6 (3.7)
	Colon	9 (4.1)	8 (4.9)
	Kidney	6 (2.7)	4 (2.5)
	Pancreas	5 (2.3)	3 (1.8)
	Sarcoma	2 (0.9)	1 (0.6)
	Other	32 (14.5)	82 (51.0)
Breslow thickness[c]	Median value (range)	0.71 (0.05–60)	-
Histopathological subtype[c]	In situ	283 (20.8)	-
	Invasive	848 (62.1)	-
	SSM	644 (47.2)	-
	NM	101 (7.4)	-
	ALM	19 (1.4)	-
	LMM	53 (3.9)	-
	Spitzoid	6 (0.4)	-
	Other	25 (1.8)	-

Abbreviations: ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; MPM, multiple primary melanoma; Na, data not available; NM, nodular melanoma; SSM, superficial spreading melanoma.

[a]Numbers do not always add up to the total due to missing data.

[b]Keratinocyte carcinomas were excluded.

[c]Histological characteristics are reported for the first melanoma.

were present in 35.1% of patients and in 26.3% of unaffected members (OR 1.5, 95% CI 1.2–2.0, $p = 0.002$). After adjustment for sex, age, country and *CDKN2A* status, carrying ≥2 variants and specifically ≥2 RHC variants remained significantly associated with melanoma (OR 1.4, 95% CI 1.0–2.0, $p = 0.046$ and OR 4.3, 95% CI 1.2–14.6, $p = 0.021$, respectively).

Overall, we identified 32 non-synonymous *MC1R* polymorphisms. V60L was the most prevalent in both patients and unaffected relatives (25.4% and 27.1%, respectively; $p = 0.331$; Table S4).

Regarding clinical characteristics, *MC1R* variants, mainly RHC substitutions, were associated with fair skin type ($p < 0.001$), and light hair colour ($p < 0.001$) in patients and unaffected relatives ($p = 0.03$ and $p = 0.003$, respectively; Table S5). Breslow thickness was higher in patients with any *MC1R* (0.73 mm) and RHC (0.73 mm) variant than in the wild-type group (0.60 mm; $p = 0.014$ and 0.046, respectively).

DISCUSSION

We report the largest multicentre cohort of melanoma-prone families with genetic data on all established high-risk genes,

TABLE 2 Type and frequency of CDKN2A variants in Mediterranean families, overall and by country.

	CDKN2A nucleotide change[a]	$p16^{INK4a}$[a]	Functional classification[b]	Greece *N* = 7 (%)	Italy *N* = 29 (%)	Spain *N* = 88 (%)	Total *N* = 124 (%)
5′ UTR	c.–25C > T	-	1/2	0	0	3 (3.4)	3 (2.4)
	c.–33G > C	-	1/2	0	2 (6.9)	0	2 (1.6)
	c.–34 G > T	-	4/5	0	0	3 (3.4)	3 (2.4)
Exon 1α	c.13G > A	p.A5T	3	0	0	1 (1.1)	1 (0.8)
	c.31C > A	p.P11T	3	0	0	1 (1.1)	1 (0.8)
	c.71G > C	p.R24P	4/5	6 (85.7)	3 (10.3)	0	9 (7.3)
	c.104G > A	p.G35E	4/5	0	0	2 (2.3)	2 (1.6)
	c.106delG	p.A36Rfs*17	4/5	0	0	6 (6.8)	6 (4.8)
	c.116A > G	p.N39S	3	0	0	1 (1.1)	1 (0.8)
	c.131insA	Y44*	4/5	0	0	1 (1.1)	1 (0.8)
	c.142C > A	p.P48T	4/5	0	6 (20.7)	0	6 (4.8)
	c.149A > G	p.Q50R	4/5	0	0	2 (2.3)	2 (1.6)
Exon 1β	c.60ins16[c]	-	4/5	0	0	1 (1.1)	1 (0.8)
	c.92C > T[c]	-	3	0	1 (3.4)	0	1 (0.8)
Intron 1/2	c.151-13T > C/c.151-18T > C	-	1/2	0	1 (3.4)	0	1 (0.8)
Exon 2	c.164G > T	p.G55V	4/5	0	0	1 (1.1)	1 (0.8)
	c.167G > T	p.S56I	4/5	0	1 (3.4)	0	1 (0.8)
	c.176T > G	p.V59G	4/5	0	1 (3.4)	9 (10.2)	10 (8.1)
	c.194T > C	p.L65P	4/5	0	1 (3.4)	1 (1.1)	2 (1.6)
	c.212A > G	p.N71S	4/5	0	1 (3.4)	5 (5.7)	6 (4.8)
	c.225_243del19	p.P75fs	4/5	0	1 (3.4)	0	1 (0.8)
	c.238C > T	p.R80*	4/5	0	0	1 (1.1)	1 (0.8)
	c.241C > T	p.P81S	4/5	0	0	1 (1.1)	1 (0.8)
	c.249C > A	p.H83Q	4/5	0	1 (3.4)	0	1 (0.8)
	c.250G > T	p.D84Y	4/5	0	0	1 (1.1)	1 (0.8)
	c.259C > T	p.R87W	4/5	0	1 (3.4)	2 (2.3)	3 (2.4)
	c.262G > T	p.E88*	4/5	0	0	1 (1.1)	1 (0.8)
	c.280_282insAG	p.L94*	4/5	0	1 (3.4)	0	1 (0.8)
	c.295C > T	p.R99W	3	0	0	1 (1.1)	1 (0.8)
	c.301G > T	p.G101W	4/5	0	6 (20.7)	28 (31.8)	34 (27.4)
	c.305C > T	p.A102V	4/5	0	0	1 (1.1)	1 (0.8)
	c.318G > A	p.V106V	1/2	0	0	1 (1.1)	1 (0.8)
	c.335G > C	p.R112P	4/5	0	0	1 (1.1)	1 (0.8)
	c.341C > T	p.P114L	4/5	0	1 (3.4)	0	1 (0.8)
	c.358delG	p.E120fs*145	4/5	0	0	4 (4.5)	4 (3.2)
	c.370C > T	p.R124C	1/2	0	0	1 (1.1)	1 (0.8)
	c.379G > T	p.A127S	1/2	0	0	2 (2.3)	2 (1.6)
	c.444C > A	p.A148T	1/2	2 (28.5)	0	0	2 (1.6)
	c.457G > A	p.D153N	4/5	0	0	2 (2.3)	2 (1.6)
Intron 2	IVS1 + 37G > C	-	1/2	0	0	2 (2.3)	2 (1.6)
	IVS2-2A > G	-	4/5	0	0	1 (1.1)	1 (0.8)
	IVS2-105A > G	-	4/5	0	0	1 (1.1)	1 (0.8)

[a]The reference sequence is NG 007485.1/NM_000077.5.

[b]Variants were functionally categorized as follows: benign, class 1; likely benign, class 2; uncertain significance, class 3; likely pathogenetic, class 4; pathogenetic, class 5. The interrogation of Varsome prediction tool and ClinVar database was performed on 31 January 2022.

[c]These genomic positions are related to $p14^{ARF}$ (NM_058195.4).

*indicates the nonsense mutation.

TABLE 3 Association of *CDKN2A* class 4–5 variants with familial clinical features, multivariate analysis.

Clinical features	Total			Italy			Spain		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Number of family members[a]									
2 (*n* = 59)	Ref			Ref			Ref		
3 (*n* = 28)	1.76	1.04–3.01	0.037	1.50	0.50–4.52	0.468	1.87	0.97–3.62	0.061
>3 (*n* = 18)	**2.56**	**1.26–5.19**	**0.009**	**6.71**	**1.98–22.74**	**0.002**	1.86	0.77–4.47	0.164
N. MPM cases in the family									
0 (*n* = 39)	Ref			Ref			Ref		
1 (*n* = 53)	4.08	2.53–6.55	<0.001	2.05	0.81–5.16	0.127	5.30	2.90–9.69	<0.001
≥2 (*n* = 13)	**8.11**	**3.33–19.74**	**<0.001**	Na[b]	Na[b]	Na[b]	**14.42**	**5.37-38.71**	**<0.001**
≥1 member with pancreatic cancer in the family									
No (*n* = 99)	Ref			Ref			Ref		
Yes (*n* = 6)	**4.79**	**2.43–9.42**	**<0.001**	**4.02**	**1.15–13.98**	**0.029**	**5.85**	**2.42–14.17**	**<0.001**

Bold values indicate the statistically significant comparisons.

Abbreviation: MPM, multiple primary melanoma.

[a] Including the proband.

[b] All five Italian families with ≥2 MPM cases were categorized in the wild type/class 1–2 group; therefore, the calculation was not feasible.

and the first study that estimates the strength of *CDKN2A* predictors in Southern European populations.

Mediterranean populations have been so far underrepresented in the studies aimed to characterize melanoma susceptibility. Our pooled analysis, including a homogeneous cohort of Southern European families, with a common geographic origin, similar lifestyle habits, skin type and exposure to environmental risk factors, offered the opportunity to identify the distinctive characteristics of hereditary melanoma in this geographic area. According to the low melanoma incidence rate in these regions,[40] families collected within the MelaNostrum Consortium were small, having mainly only two affected members and first-degree relatives. About 30% of them had MPM-affected members, underlining the importance of multiple primaries in the familial setting.

The frequency of *CDKN2A* variants in families with 2, 3 or more affected members has been estimated at 4%, 8% and 38%, respectively, in previous studies (reviewed in 41). A combined analysis of family studies from three continents identified mutation rates from 20% in Australia, to 45% in North America and 57% in Europe in families with at least three affected members.[16,42] We observed an overall frequency of pathogenic variants in our families of 13.8%, with the lowest in Italy (10.6%) and the highest in Greece (42.9%). Although the low number of Greek families might have contributed to amplifying these differences, the mutation rates identified in our families are consistent with those previously reported in each country, excluding reports of higher frequency due to founder effects.[15,43–48] A higher impact of genetic risk factors has been suggested in the Greek low incidence population.[48]

A high number of affected members, early age at diagnosis and MPM are considered predictors of *CDKN2A* variation.[12,35,37,49,50] However, the effects of predictors vary widely among geographic regions, being more stringent in high-incidence countries as Australia (≥2 MPM patients, age at diagnosis ≤40 and ≥6 affected in a family) than in moderate-incidence area as Northern Europe (≥1 MPM patients, ≥4 affected, age at diagnosis ≤50 and ≥1 patient with pancreatic cancer) and North America (≥1 MPM and age ≤ 40 years).[12] Four regression models have been published for these regions to estimate the probability of a germline *CDKN2A* mutation but they may be inappropriate for other populations.[51–54] We identified a prediction algorithm specific for Mediterranean melanoma-prone families with the occurrence of ≥2 MPM patients and pancreatic cancer being the strongest predictors of *CDKN2A* variants, followed by the presence of more than three affected members. Stratifying by country, we identified a different strength of association for these factors, which might help select patients for genetic testing across the regions.

In *CDKN2A*- and *CDK4*-negative families, 3.8% carried *POT1* variations, with the most frequent change being the likely pathogenetic S270N, only found in Italian families due to the founder effect.[27] More recently, the presence of *POT1* variants has been associated with a high number of nevi, Spitzoid morphology of the melanoma and occurrence of other cancers in the family.[6,55] Common variants in this gene have been identified as risk factors for sporadic melanoma in a large melanoma-metanalysis GWAS.[32] In our *POT1*-mutated families, we observed increased melanoma thickness in carriers. Variants in *TERF2IP* and *ACD* were less frequent in our families (2.5% and 0.8%, respectively), while none carried the pathogenic −57 T > G change in the *TERT* promoter or the hot spot *CDK4* variant.

A higher prevalence of *MC1R* polymorphisms were identified in patients than in their unaffected relatives, consistent with individual studies from Southern European

TABLE 4 Type and frequency of variants in *ACD*, *BAP1*, *POT1*, *TERF2IP* and *TERT* genes, in families as a whole and by country.

Genes	Variants	Functional classification[a]	Total families	Italy	Spain
ACD			***N*** **= 360 (%)**[b]	***N*** **= 226 (%)**[b]	***N*** **= 134 (%)**[b]
	Total		**3 (0.8)**	**1 (0.4)**	**2 (1.5)**
	D392fs	4/5	1 (0.3)	0	1 (0.7)
	S407L	1/2	2 (0.5)	1 (0.4)	1 (0.7)
BAP1			***N*** **= 391 (%)**[b]	***N*** **= 226 (%)**[b]	***N*** **= 165 (%)**[b]
	Total		**1 (0.3)**	0	**1 (0.6)**
	S455P	1/2	1 (0.3)	0	1 (0.6)
POT1			***N*** **= 363 (%)**[b]	***N*** **= 227 (%)**[b]	***N*** **= 136 (%)**[b]
	Total		**14 (3.8)**	**8 (3.5)**	**6 (4.4)**
	I78T	3	1 (0.3)	0	1 (0.7)
	125_255del	4/5	1 (0.3)	0	1 (0.7)
	A135T	3	1 (0.3)	1 (0.4)	0
	R137H	3	1 (0.3)	1 (0.4)	0
	S270N	4/5	5 (1.4)	5 (2.2)	0
	D313N	3	1 (0.3)	0	1 (0.7)
	E344*	4/5	1 (0.3)	0	1 (0.7)
	D598N	4/5	2 (0.5)	0	2 (1.5)
	Q623H	4/5	1 (0.3)	1 (0.4)	0
TERF2IP			***N*** **= 358 (%)**[b]	***N*** **= 226 (%)**[b]	***N*** **= 132 (%)**[b]
	Total		**9 (2.5)**	**4 (1.8)**	**4 (3.0)**
	S206Y	1/2	2 (0.6)	2 (0.8)	0
	E237Q	3	1 (0.3)	1 (0.4)	0
	T346A	3	1 (0.3)	0	1 (0.7)
	T346I	3	1 (0.3)	0	1 (0.7)
	A348S	3	2 (0.6)	1 (0.4)	1 (0.7)
	L350V	3	1 (0.3)	0	1 (0.7)
	Q354*	4/5	1 (0.3)	0	1 (0.7)
TERT			***N*** **= 380 (%)**[b]	***N*** **= 222 (%)**[b]	***N*** **= 158 (%)**[b]
	Total		**2 (0.5)**	**2 (0.9)**	**0**
	G424S	3	1 (0.3)	1 (0.4)	0
	I729V	3	1 (0.3)	1 (0.4)	0

Bold values indicate the statistically significant comparisons.

[a] Variants were functionally categorized as follows: benign, class 1; likely benign, class 2; uncertain significance, class 3; likely pathogenetic, class 4; pathogenetic, class 5.

[b] Families negative for *CDKN2A* class 3–5 variants, tested for each gene at the Division of Cancer Epidemiology and Genetics, National Cancer Institute, and included in this study.

countries.[15,34,47,56–60] In our cohort, RHC variants were associated with a fair phenotype, supporting of *MC1R* as an important determinant of human pigmentation.

This study has some limitations. We aimed at covering the Southern European populations with Italy and Spain contributing with most families, while Greece and other countries were poorly or not represented. Data from other Mediterranean countries such as Croatia, Southern France and Cyprus that only recently joined the MelaNostrum Consortium will be analysed in the future. Although this study includes a proportion of melanoma families already published in different single manuscripts (reported in the Table S1), our pooled analysis allowed to uniformly analyse clinical and genetic data, overcoming the heterogeneity reported across previous findings, and to identify the characteristics of hereditary melanoma specific for the Southern European population, in general. *CDKN2A* variation frequency for both the entire cohort and for Italy might have been underestimated, since we had to exclude Genoa families (only *CDKN2A*-negative families were included in the Consortium) from the calculation. Finally, this study collected genetic data obtained by different methodologies. However, for CDKN2A, CDK4 and MC1R data, which were obtained from targeted sequencing at each centre, but the high level of methodological standardization for the analysis of these two genes implemented

within the Consortium reduced any possible technical bias. For other high-penetrance genes, genetic data were obtained from whole exome sequencing centrally performed at the NCI. In addition, to reduce variant misclassification we performed a stringent categorization that was reviewed by three experts.

In conclusion, our study provides a complete description of genetic susceptibility in melanoma-prone families from Southern Europe, further confirming the importance of Consortium-based research for studies of rare susceptibility genes. We estimated the strength of *CDKN2A* predictors in Mediterranean countries, which might be useful for genetic counselling processes and preventive strategies in the Mediterranean population.

ACKNOWLEDGEMENTS

Samples from IVO were retrieved from the Biobank of the Instituto Valenciano de Oncología. We are indebted to patients and families whose generosity and cooperation made this study possible.

FUNDING INFORMATION

The research at the Melanoma Unit at the Hospital Clinic Barcelona was partially financed by the following grants: PI15/00716, PI15/00956 and PI18/00419 from Fondo de Investigaciones Sanitarias (Spain), by the CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain, co-funded by ISCIII-Subdireccion General de Evaluacion and European Regional Development Fund (ERDF), 'a way to make Europe'; AGAUR 2017_SGR_1134 and CERCA Programme by Generalitat de Catalunya, Spain; European Commission under the 6th Framework Programme, Contract No. LSHC-CT-2006-018702 (GenoMEL), by the European Commission under the 7th Framework Programme, Diagnoptics, and the European Commission under the HORIZON2020 Framework Programme, iTobos and Qualitop; The National Cancer Institute (NCI) of the US National Institute of Health (NIH) (CA83115); 'Fundació La Marató de TV3' 201331-30, Catalonia, Spain; 'Fundación Científica de la Asociación Española Contra el Cáncer' GCB15152978SOEN, Spain. Part of the work was carried out at the Esther Koplowitz Center, Barcelona. The research at the Ospedale Policlinico San Martino, Genetica dei Tumori rari and University of Genoa was partially financed by Italian Ministry of Health [grant number RF-2016-02362288 to P.G.] and Ricerca Corrente/5 × 1000 to IRCCS Ospedale Policlinico San Martino to P.G. The research at National Cancer Institute, USA, was financed by Intramural Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH.

CONFLICT OF INTEREST STATEMENT

P. Aguilera has received honoraria for lectures by ISDIN. C. Carrera obtained honoraria for lectures by ISDIN. MC. Fargnoli has served on advisory boards and received honoraria for lectures by BMS, MSD, Novartis. J. Malvehy served as advisory board member, consultant, speaker, and received grants and support from Almirall, Amgen S.A, Athena Tech, Bioderma, Bristol, Canfield, Damae, Diagnosis Dermatologica, ISDIN, Leo Messi Foundation, MSD, La Roche Posay, Pfizer, Regeneron, Sun pharma and Sanofi. M. Potrony received honoraria for lectures and support for attending meeting by ISDIN. S. Puig has been principal investigator in clinical trials, served as consultant and paid speaker and received grants and support from Almirall, Amgen S.A, Athena Tech, Bioderma, Bristol, Canfield, Damae, ISDIN, Mayne Pharma, MSD, La Roche Posay, Pfizer, Regeneron Pharmaceuticals, SYNTERACT, Sun pharma, Sanofi and TRIAL FORM SUPPORT, S.L. A. Stratigos has received grants and/or honoraria from Abbvie, BMI, Novartis, Regeneron, Genesis Pharma, Janssen Cilag, Regeneron, Sanofi. All other authors declared that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Patients included in the study are participants in local protocols approved by Institutional Review Bboards (IRB) at each centre, as reported in the Appendix S1. Informed consent was obtained from participants at each centre.

ORCID

C. Pellegrini https://orcid.org/0000-0003-2168-8097
M. Potrony https://orcid.org/0000-0003-2766-0765
I. Stefanaki https://orcid.org/0000-0002-2964-370X
L. Di Nardo https://orcid.org/0000-0002-2087-7526
C. Carrera https://orcid.org/0000-0003-1608-8820
K. Peris https://orcid.org/0000-0003-1957-6600
E. Nagore https://orcid.org/0000-0003-3433-8707
S. Puig https://orcid.org/0000-0003-1337-9745
MC. Fargnoli https://orcid.org/0000-0002-7249-2556

REFERENCES

1. Stratigos AJ, Fargnoli MC, De Nicolo A, Peris K, Puig S, Soura E, et al. MelaNostrum: a consensus questionnaire of standardized epidemiologic and clinical variables for melanoma risk assessment by the MelaNostrum consortium. J Eur Acad Dermatol Venereol. 2018;32(12):2134–41. https://doi.org/10.1111/jdv.15208
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49. https://doi.org/10.3322/caac.21660
3. Shekar SN, Duffy DL, Youl P, Baxter AJ, Kvaskoff M, Whiteman DC, et al. A population-based study of Australian twins with melanoma suggests a strong genetic contribution to liability. J Invest Dermatol. 2009;129(9):2211–9. https://doi.org/10.1038/jid.2009.48
4. Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, et al. Familial risk and heritability of cancer among twins in Nordic countries. JAMA. 2016;315(1):68–76. https://doi.org/10.1001/jama.2015.17703
5. Visser M, van der Stoep N, Gruis N. Progress report on the major clinical advances in patient-oriented research into familial melanoma (2013–2018). Fam Cancer. 2019;18(2):267–71. https://doi.org/10.1007/s10689-018-00115-3

6. Ribeiro Moura Brasil Arnaut J, Dos Santos Guimarães I, Evangelista Dos Santos AC, de Moraes Lino da Silva F, Ricardo Machado J, de Melo AC. Molecular landscape of hereditary melanoma. Crit Rev Oncol Hematol. 2021;164:103425. https://doi.org/10.1016/j.critrevonc.2021
7. Olsen CM, Carroll HJ, Whiteman DC. Familial melanoma: a meta-analysis and estimates of attributable fraction. Cancer Epidemiol Biomarkers Prev. 2010;19(1):65–73. https://doi.org/10.1158/1055-9965
8. Chen T, Hemminki K, Kharazmi E, Ji J, Sundquist K, Fallah M. Multiple primary (even in situ) melanomas in a patient pose significant risk to family members. Eur J Cancer. 2014;50(15):2659–67. https://doi.org/10.1016/j.ejca.2014.07.007
9. Tucker MA, Elder DE, Curry M, Fraser MC, Pichler V, Zametkin D, et al. Risks of melanoma and other cancers in melanoma-prone families over 4 decades. J Invest Dermatol. 2018;138(7):1620–6. https://doi.org/10.1016/j.jid.2018.01.021
10. Frank C, Sundquist J, Hemminki A, Hemminki K. Risk of other cancers in families with melanoma: novel familial links. Sci Rep. 2017;15(7):42601. https://doi.org/10.1038/srep42601
11. Dalmasso B, Ghiorzo P. Evolution of approaches to identify melanoma missing heritability. Expert Rev Mol Diagn. 2020;20(5):523–31. https://doi.org/10.1080/14737159.2020.1738221
12. Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Timothy Bishop D, et al. Features associated with germline *CDKN2A* mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet. 2007;44(2):99–106. https://doi.org/10.1136/jmg.2006.043802
13. Puig S, Potrony M, Cuellar F, Puig-Butille JA, Carrera C, Aguilera P, et al. Characterization of individuals at high risk of developing melanoma in Latin America: bases for genetic counseling in melanoma. Genet Med. 2016;18(7):727–36. https://doi.org/10.1038/gim.2015.160
14. Mangas C, Potrony M, Mainetti C, Bianchi E, Carrozza Merlani P, Mancarella Eberhardt A, et al. Genetic susceptibility to cutaneous melanoma in southern Switzerland: role of *CDKN2A*, *MC1R* and *MITF*. Br J Dermatol. 2016;175(5):1030–7. https://doi.org/10.1111/bjd.14897
15. Huerta C, Garcia-Casado Z, Bañuls J, Moragon M, Oliver V, Unamuno B, et al. Characteristics of familial melanoma in Valencia, Spain, based on the presence of *CDKN2A* mutations and *MC1R* variants. Acta Derm Venereol. 2018;98(5):512–6. https://doi.org/10.2340/00015555-2898
16. Goldstein AM, Chan M, Harland M, Gillanders EM, Hayward NK, Avril MF, et al. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. Cancer Res. 2006;66(20):9818–28. https://doi.org/10.1158/0008-5472.CAN-06-0494
17. Aitken J, Welch D, Duffy A, Milligan A, Green A, Martin N, et al. *CDKN2A* variants in a population-based sample of Queensland families with melanoma. J Natl Cancer Inst. 1999;91(5):446–52. https://doi.org/10.1093/jnci/91.5.446
18. Loo JC, Liu L, Hao A, Agatep R, Shennan M, Summers A, et al. Germline splicing mutations of *CDKN2A* predispose to melanoma. Oncogene. 2003;22(41):6387–94. https://doi.org/10.1038/sj.onc.1206736
19. Begg CB, Hummer A, Mujumdar U, Armstrong BK, Kricker A, Marrett LD, et al. Familial aggregation of melanoma risks in a large population-based sample of melanoma cases. Cancer Causes Control. 2004;15(9):957–65. https://doi.org/10.1007/s10522-004-2474-2
20. Begg CB, Orlow I, Hummer AJ, Armstrong BK, Kricker A, Marrett LD, et al. Lifetime risk of melanoma in *CDKN2A* mutation carriers in a population-based sample. J Natl Cancer Inst. 2005;97(20):1507–15. https://doi.org/10.1093/jnci/dji312
21. Bruno W, Ghiorzo P, Battistuzzi L, Ascierto PA, Barile M, Gargiulo S, et al. Clinical genetic testing for familial melanoma in Italy: a cooperative study. J Am Acad Dermatol. 2009;61(5):775–82. https://doi.org/10.1016/j.jaad.2009.03.039
22. Taylor NJ, Handorf EA, Mitra N, Avril MF, Azizi E, Bergman W, et al. Phenotypic and histopathological tumor characteristics according to *CDKN2A* mutation status among affected members of melanoma families. J Invest Dermatol. 2016;136(5):1066–9. https://doi.org/10.1016/j.jid.2016.01.009
23. Puig S, Marcoval J, Paradelo C, Azon A, Bartralot R, Bel S, et al. Melanoma incidence increases in the elderly of Catalonia but not in the younger population: effect of prevention or consequence of immigration? Acta Derm Venereol. 2015;95(4):422–6. https://doi.org/10.2340/00015555-1997
24. Potrony M, Badenas C, Aguilera P, Puig-Butille JA, Carrera C, Malvehy J, et al. Update in genetic susceptibility in melanoma. Ann Transl Med. 2015;3(15):210. https://doi.org/10.3978/j.issn.2305-5839.2015.08.11
25. Puntervoll HE, Yang XR, Vetti HH, Bachmann IM, Avril MF, Benfodda M, et al. Melanoma prone families with *CDK4* germline mutation: phenotypic profile and associations with *MC1R* variants. J Med Genet. 2013;50(4):264–70. https://doi.org/10.1136/jmedgenet-2012-101455
26. Robles-Espinoza CD, MdelC V-H, Hayward NK, Adams DJ. Telomere-regulating genes and the telomere interactome in familial cancers. Mol Cancer Res. 2015;13(2):211–22. https://doi.org/10.1158/1541-7786.MCR-14-0305
27. Shi J, Yang XR, Ballew B, Rotunno M, Calista D, Fargnoli MC, et al. Rare missense variants in *POT1* predispose to familial cutaneous malignant melanoma. Nat Genet. 2014;46(5):482–6. https://doi.org/10.1038/ng.2941
28. Aoude LG, Pritchard AL, Robles-Espinoza CD, Wadt K, Harland M, Choi J, et al. Nonsense mutations in the shelterin complex genes *ACD* and *TERF2IP* in familial melanoma. J Natl Cancer Inst. 2014;107(2):dju408. https://doi.org/10.1093/jnci/dju408
29. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. *TERT* promoter mutations in familial and sporadic melanoma. Science. 2013;22:959–61. https://doi.org/10.1126/science.1230062
30. O'Shea SJ, Robles-Espinoza CD, McLellan L, Harrigan J, Jacq X, Hewinson J, et al. A population-based analysis of germline *BAP1* mutations in melanoma. Hum Mol Genet. 2017;26(4):717–28. https://doi.org/10.1093/hmg/ddw403
31. Walpole S, Pritchard AL, Cebulla CM, Pilarski R, Stautberg M, Davidorf FH, et al. Comprehensive study of the clinical phenotype of germline BAP1 variant-carrying families worldwide. J Natl Cancer Inst. 2018;110(12):1328–41. https://doi.org/10.1093/jnci/djy171
32. Landi MT, Bishop DT, MacGregor S, Machiela MJ, Stratigos AJ, Ghiorzo P, et al. Genome-wide association meta-analyses combining multiple risk phenotypes provide insights into the genetic architecture of cutaneous melanoma susceptibility. Nat Genet. 2020;52(5):494–504. https://doi.org/10.1038/s41588-020-0611-8
33. Zanna I, Caini S, Raimondi S, Saieva C, Masala G, Massi D, et al. Germline *MC1R* variants and frequency of somatic *BRAF*, *NRAS*, and *TERT* mutations in melanoma: literature review and meta-analysis. Mol Carcinog. 2021;60(3):167–71. https://doi.org/10.1002/mc.23280
34. Fargnoli MC, Gandini S, Peris K, Maisonneuve P, Raimondi S. *MC1R* variants increase melanoma risk in families with *CDKN2A* mutations: a meta-analysis. Eur J Cancer. 2010;46(8):1413–20. https://doi.org/10.1016/j.ejca.2010.01.027
35. Cust AE, Harland M, Makalic E, Schmidt D, Dowty JG, Aitken JF, et al. Melanoma risk for *CDKN2A* mutation carriers who are relatives of population-based case carriers in Australia and the UK. J Med Genet. 2011;48(4):266–72. https://doi.org/10.1136/jmg.2010.086538
36. Maubec E, Chaudru V, Mohamdi H, Blondel C, Margaritte-Jeannin P, Forget S, et al. Familial melanoma: clinical factors associated with germline *CDKN2A* mutations according to the number of patients affected by melanoma in a family. J Am Acad Dermatol. 2012;67(6):1257–64. https://doi.org/10.1016/j.jaad.2012.05.014
37. Harland M, Cust AE, Badenas C, Chang YM, Holland EA, Aguilera P, et al. Prevalence and predictors of germline *CDKN2A* mutations for melanoma cases from Australia, Spain and the United Kingdom. Hered Cancer Clin Pract. 2014;12(1):20. https://doi.org/10.1186/1897-4287-12-20
38. Richards S, Aziz N, Bale S, Bick D, das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a

joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24. https://doi.org/10.1038/gim.2015.30
39. Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Melanocortin 1 receptor variants: functional role and pigmentary associations. Photochem Photobiol. 2011;87(5):978–87. https://doi.org/10.1111/j.1751-1097.2011.00970.x
40. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer; 2022. Available from: https://gco.iarc.fr/today, Accessed 8 March 2022.
41. Leachman SA, Carucci J, Kohlmann W, Banks KC, Asgari MM, Bergman W, et al. Selection criteria for genetic assessment of patients with familial melanoma. J Am Acad Dermatol. 2009;61(4):677.e1–14. https://doi.org/10.1016/j.jaad.2009.03.016
42. Bishop DT, Demenais F, Goldstein AM, Bergman W, Bishop JN, Bressac-de Paillerets B, et al. Geographical variation in the penetrance of *CDKN2A* mutations for melanoma. J Natl Cancer Inst. 2002;94(12):894–903. https://doi.org/10.1093/jnci/94.12.894
43. Potrony M, Puig-Butillé JA, Aguilera P, Badenas C, Carrera C, Malvehy J, et al. Increased prevalence of lung, breast, and pancreatic cancers in addition to melanoma risk in families bearing the cyclin-dependent kinase inhibitor 2A mutation: implications for genetic counseling. J Am Acad Dermatol. 2014;71(5):888–95. https://doi.org/10.1016/j.jaad.2014.06.036
44. Mantelli M, Barile M, Ciotti P, Ghiorzo P, Lantieri F, Pastorino L, et al. High prevalence of the G101W germline mutation in the *CDKN2A* (P16(ink4a)) gene in 62 Italian malignant melanoma families. Am J Med Genet. 2002;107(3):214–21. https://doi.org/10.1002/ajmg.10137
45. Bruno W, Pastorino L, Ghiorzo P, Andreotti V, Martinuzzi C, Menin C, et al. Multiple primary melanomas (MPMs) and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian melanoma intergroup. J Am Acad Dermatol. 2016;74(2):325–32. https://doi.org/10.1016/j.jaad.2015.09.053
46. Márquez-Rodas I, González MM, Nagore E, et al. Frequency and characteristics of familial melanoma in Spain: the FAM-GEM-1 study. PLoS ONE. 2015;10(4):e0124239. https://doi.org/10.1371/journal.pone.0124239
47. Pellegrini C, Maturo MG, Martorelli C, Suppa M, Antonini A, Kostaki D, et al. Characterization of melanoma susceptibility genes in high-risk patients from Central Italy. Melanoma Res. 2017;27(3):258–67. https://doi.org/10.1097/CMR.0000000000000323
48. Karagianni F, Njauw CN, Kypreou KP, Stergiopoulou A, Plaka M, Polydorou D, et al. CDKN2A/CDK4 status in Greek patients with familial melanoma and association with Clinico-epidemiological parameters. Acta Derm Venereol. 2018;98(9):862–6. https://doi.org/10.2340/00015555-2969
49. Potjer TP, van der Stoep N, Houwing-Duistermaat JJ, Konings ICAW, Aalfs CM, van den Akker PC, et al. Pancreatic cancer-associated gene polymorphisms in a nation-wide cohort of p16-Leiden germline mutation carriers; a case-control study. BMC Res Notes. 2015;8:264. https://doi.org/10.1186/s13104-015-1235-4
50. Bruno W, Dalmasso B, Barile M, Andreotti V, Elefanti L, Colombino M, et al. Predictors of germline status for hereditary melanoma: 5 years of multi-gene panel testing within the Italian melanoma intergroup. ESMO Open. 2022;7(4):100525. https://doi.org/10.1016/j.esmoop.2022.100525
51. Niendorf KB, Goggins W, Yang G, Tsai KY, Shennan M, Bell DW, et al. MELPREDICT: a logistic regression model to estimate *CDKN2A* carrier probability. J Med Genet. 2006;43(6):501–6. https://doi.org/10.1136/jmg.2005.032441
52. Wang W, Niendorf KB, Patel D, Blackford A, Marroni F, Sober AJ, et al. Estimating *CDKN2A* carrier probability and personalizing cancer risk assessments in hereditary melanoma using MelaPRO. Cancer Res. 2010;70(2):552–9. https://doi.org/10.1158/0008-5472.CAN-09-2653
53. Potjer TP, Helgadottir H, Leenheer M, van der Stoep N, Gruis NA, Höiom V, et al. CM-score: a validated scoring system to predict *CDKN2A* germline mutations in melanoma families from northern Europe. J Med Genet. 2018;55(10):661–8. https://doi.org/10.1136/jmedgenet-2017-105205
54. Taylor NJ, Mitra N, Qian L, Avril MF, Bishop DT, Bressac-de Paillerets B, et al. Estimating *CDKN2A* mutation carrier probability among global familial melanoma cases using GenoMELPREDICT. J Am Acad Dermatol. 2019;81(2):386–94. https://doi.org/10.1016/j.jaad.2019.01.079
55. Sargen MR, Calista D, Elder DE, Massi D, Chu EY, Potrony M, et al. Histologic features of melanoma associated with germline mutations of *CDKN2A*, *CDK4*, and *POT1* in melanoma-prone families from the United States, Italy, and Spain. J Am Acad Dermatol. 2020;83(3):860–9. https://doi.org/10.1016/j.jaad.2020.03.100
56. Landi MT, Kanetsky PA, Tsang S, Gold B, Munroe D, Rebbeck T, et al. MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. J Natl Cancer Inst. 2005;97(13):998–1007. https://doi.org/10.1093/jnci/dji176
57. Stratigos AJ, Dimisianos G, Nikolaou V, Poulou M, Sypsa V, Stefanaki I, et al. Melanocortin receptor-1 gene polymorphisms and the risk of cutaneous melanoma in a low-risk southern European population. J Invest Dermatol. 2006;126:1842–9. https://doi.org/10.1038/sj.jid.570029
58. Demenais F, Mohamdi H, Chaudru V, Goldstein AM, Newton Bishop JA, Bishop DT, et al. Association of *MC1R* variants and host phenotypes with melanoma risk in CDKN2A mutation carriers: a GenoMEL study. J Natl Cancer Inst. 2010;102(20):1568–83. https://doi.org/10.1093/jnci/djq363
59. De Torre C, Garcia-Casado Z, Martínez-Escribano JA, Botella-Estrada R, Bañuls J, Oliver V, et al. Influence of loss of function *MC1R* variants in genetic susceptibility of familial melanoma in Spain. Melanoma Res. 2010;20(4):342–8. https://doi.org/10.1097/CMR.0b013e32833b159d
60. Ghiorzo P, Bonelli L, Pastorino L, Bruno W, Barile M, Andreotti V, et al. *MC1R* variation and melanoma risk in relation to host/clinical and environmental factors in *CDKN2A* positive and negative melanoma patients. Exp Dermatol. 2012;21(9):718–20. https://doi.org/10.1111/j.1600-0625.2012.01549

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pellegrini C, Cardelli L, Ghiorzo P, Pastorino L, Potrony M, García-Casado Z, et al. High- and intermediate-risk susceptibility variants in melanoma families from the Mediterranean area: A multicentre cohort from the MelaNostrum Consortium. J Eur Acad Dermatol Venereol. 2023;37:2498–2508. https://doi.org/10.1111/jdv.19461