Dermatology Practical & Conceptual

Prevalence of *CDKN2A*, *CDK4*, *POT1*, *BAP1*, *MITF*, *ATM*, and *TERT* Pathogenic Variants in a Single-Center Retrospective Series of Patients With Melanoma and Personal or Family History Suggestive of Genetic Predisposition

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ABSTRACT

Introduction: Approximately 20%-45% of familial melanoma (FM) cases are associated with genetic predisposition.

Objectives: This single-center retrospective study aimed to assess the frequency of pathogenic variants (PV) in the main melanoma-predisposing genes in patients with cutaneous melanoma and investigate the clinical predictors of genetic predisposition.

Methods: Patients included were those diagnosed with cutaneous melanoma at the Dermatology Unit of the University Hospital of Verona, Italy, from 2000 to 2022, presenting at least one of the followings: multiple melanomas (≥ 3); personal/family history of pancreatic cancer (PC) (up to 2nd-degree relatives); ≥ 2 1st-degree relatives with melanoma; ≥ 1 1st-degree relatives with early-onset (<45 years) melanoma and tested for *CDKN2A*, *CDK4*, *POT1*, *BAP1*, *MITF*, *ATM*, and *TERT*.

Results: During the study period, 35 out of 1320 patients (2.7%) underwent genetic testing. Four patients (11.4%) harbored a PV in a melanoma-predisposing gene, three in *CDKN2A* (8.6%), and one in *MITF* (2.9%). Variants currently classified as being of unknown clinical significance (VUS) were detected in *CDKN2A* (N = 1), *MITF* (N = 1), and *ATM* (N = 2). Family history of PC and ≥5 melanomas, personal history of ≥50 nevi, and ≥4 melanomas were significantly associated with PV in tested genes ($P < 0.05$).

Conclusions: The prevalence of PV in predisposing genes in FM was lower than previously reported in Italian registries. Possible reasons include deleterious variants in untested intermediate/low-penetrance genes or yet-to-be-discovered high-penetrance genes and environmental risk factors. A family history of PC, a high number of nevi and melanomas predict a monogenic predisposition to melanoma.

Introduction

Melanoma is a skin cancer that originates from the neoplastic transformation of melanocytes. Previously considered a rare diagnosis, its incidence has grown worldwide in recent decades [1,2].

According to the patient family history, melanoma can be classified as sporadic or familial (FM). Randomly acquired genomic changes in melanocytes resulting from environmental factors or aging and favored by certain phenotypic traits (eg skin color) cause sporadic melanoma [1]. Approximately 8% to 12% of patients with melanoma have at least one first-degree relative who developed this cancer [1]. Most familial aggregations of melanoma are not related to a monogenic inheritance. They can be associated with grouping sporadic cases in families with common risk factors, such as sun exposure or fair skin [1]. In up to 45% of cases, patients with FM carry a PV in a predisposing gene [3]. In this context, fewer somatic acquired mutations are likely required before a critical level for oncogenesis is achieved.

Prevalence of moderate or high-risk alleles and penetrance of PV in these genes vary from 15% to 45% according to geographical area and ethnicity [3-7], which is likely a reflection of a combination of environmental factors with several other inheritable genetic modifiers shared by family members. Considering the distribution of polymorphic low-risk alleles across geographical areas, no single guideline for melanoma genetic testing can be adopted worldwide. However, in Southern European populations, where the incidence of melanoma is historically considered low, genetic testing is generally proposed in the presence of melanoma in at least two first-degree relatives or three second-degree relatives in the same branch of the family [8].

Since the mid-1990s, many melanoma predisposition genes have been identified. *CDKN2A* is involved in approximately 20%-40% of familial melanoma cases [9]. The *CDKN2A* gene consists of three exons encoding two proteins derived from alternatively framed transcripts, p16 (p16INK4A) and p14 (p14ARF), both acting as a tumor suppressor involved in cell cycle control.

Genome-wide DNA sequencing studies have demonstrated that the *CDKN2A* locus is in a nevus-associated genomic region. Carriers of *CDKN2A* PV show a significantly higher number of nevi and clinically atypical nevi than non-carriers [10, 11]. Some, but not all, *CDKN2A* carriers exhibit a clinical phenotype consistent with atypical multiple mole melanoma syndromes (FAMMM) [5,12,13].

The detection rate of *CDKN2A* PV varies across different geographical areas [4]. In 2009, *CDKN2A* PV were identified in about 33% of FM cases in Italy and 25% of families with only two affected first-degree relatives [13]. Recently, a large multicentric Italian study showed a lower detection rate, identifying 21 carriers of *CDKN2A* PV out of 373 melanoma patients having one first-degree relative with melanoma (5.63%) and ten carriers out of 101 patients with two first-degree relatives (9.9%) [14].

Increasing evidence suggests melanoma is part of larger cancer syndromes [9]. *CDKN2A* PV affecting p16INK4A are associated with an increased risk of developing pancreatic cancer (PC), up to 20% within the age of 70 [15,16].

PV in other genes such as *CDK4*, *POT1*, *BAP1*, *MITF*, *ATM* and *TERT* have been associated to moderate to high-risk to develop melanoma, as well as other malignancies, and are part of multi-gene panels employed in clinic to assess the presence of an inherited melanoma predisposition syndrome [3,8,17]. The prevalence of PV in these genes in Italy is as high as 3.7% in families with at least two first-degree relatives with melanoma and 4% with three affected first-degree relatives [14].

Objectives

This study aims to estimate the prevalence of germline PV in *CDKN2A*, *CDK4*, *POT1*, *BAP1*, *MITF*, *ATM*, and *TERT* and to evaluate the impact of patient's cutaneous phenotype and personal/family history of PC and melanomas as

predictors of the presence of a monogenic predisposition in a single-center cohort of highly selected Italian melanoma patients. Taking into account the rising of melanoma incidence and the recent decrease in the prevalence of PV among FM and multiple primary melanoma cases in Italy, to select the study population we employed criteria that are more stringent than those used in the current clinical practice to propose genetic counseling and testing in Italy (Italian societies such as Italian Society of Human Genetics (SIGU) [18], Italian Society of Dermatology (SIDeMaST) [19] and Italian Society of Oncology (AIOM) [20]).

Methods

Study Population and Ethics Statement

Medical records of 1320 patients diagnosed with cutaneous melanoma at the Dermatology Unit of the University of Verona Hospital Trust, Verona, Italy, from April 2000 to March 2022 were retrospectively reviewed.

To select patients to address to genetic counseling, we employed criteria that mirror those used in countries with a high incidence of melanoma, such as Sweden [21] paying attention to the number of melanoma in case of patients with multiple or patients with a positive family history of melanoma, to the age at diagnosis and to family history of PC.

Patients with one or more of the following criteria were identified:

- Multiple melanomas (≥ 3)
- Personal/family history for PC (up to the second degree of kinship)
- ≥ 2 melanomas among first-degree relatives
- First-degree relatives with early-onset melanoma (< 45 years)

Forty-six patients (3.5%) met the selection criteria, and 35 (76.1%) agreed to undergo genetic counseling and testing for the main melanoma predisposition genes and were included in this study.

The study protocol was approved by the local Institutional Review Board "Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo" on 19th of April 2023 (Prot. n. 24955, 26th of April 2023) and it was in agreement with the principles of the Declaration of Helsinki. All patients provided a signed informed consent approved by a local Institutional Review Board. For each patient, the diagnosis of melanoma was confirmed by histopathological analysis.

Data Collected

The following patient data were collected: environmental risk factors (eg sun exposure, history of burns), personal history of cancer (any), family history of cancer (any), including the elaboration of a three-generations family tree. Genetic counseling and testing were centralized (Medical Genetics Unit, IRCCS Sacro Cuore Don Calabria Hospital in Negrar di Valpolicella, Verona) for 29 patients (82.9%). Six patients (17.1%) were referred to other oncogenetic centers for geographical reasons.

Genetic Analysis

Genetic testing was performed on DNA extracted from the patient peripheral blood. Genetic testing provided in IRCCS Sacro Cuore Don Calabria Hospital in Negrar di Valpolicella, Varona analyzed *CDKN2A*, *CDK4*, *POT1*, *BAP1*, *MITF*, *ATM*, and *TERT*. Mutational analysis was performed by using massive parallel sequencing with Thermo Fisher Scientific S5 Prime platform, with multiplex amplification of all exons and exon-intron junctions up to 20bp by a custom Ampliseq On-Demand Panel IAD1965574_1000 (Thermo Fisher Scientific). The reads obtained were aligned on reference genome GRCh37/hg19. Data analysis and variants annotation was performed using two distinct pipelines: 1) Ion Torrent Suite v5.12, Ion-Reporter v5.12 (Thermo Fisher Scientific); 2) Ensembl Variant Effect Predictor (EMBL-EBI, Wellcome Sanger Institute). Visualization of the reads performed with IGV 2.8 (Broad Institute). Search for large deletions/insertions (Copy Number Variation, CVN) of the genes included in the panel performed by CNVs analysis with Ion Reporter v5.12 (Thermo Fisher Scientific).

Genetic testing performed in other centers investigated a larger amplicon-based gene panel sequenced using Thermo Fisher Scientific S5 Prime platform analogous to the one of our reference lab. The presence of the seven genes analyzed at the Sacro Cuore Don Calabria Hospital panel was considered mandatory to include the cases in the study cohort.

Reference databases employed for clinical interpretation of gene variants were: ClinVar, LOVD database (LOVD v.3.0 Build 29 - LOVD software ©2004-2023), Varsome and Franklin by Genoox.

Statistical Analysis

Median and interquartile range were used to present descriptive statistics. The association between the presence of PV in melanoma predisposition genes and various clinical characteristics was assessed using the Chi-square or Fisher exact test. The study utilized a two-sided Fisher exact test in the SPSS software to determine the statistical association. Statistical significance was set at a P value threshold of less than 0.05. Statistical analyses were performed using SPSS software ver. 25 (IBM)

Results

Genetic Testing Results

Genetic testing revealed the presence of hereditary melanoma in 4 out of 35 index cases (11.4%). In particular, three *CDKN2A* (8.6%) and one *MITF* (2.9%) PV were identified in four distinct patients. Moreover, variants currently classified as VUS were detected in *CDKN2A* (N = 1), *MITF* (N = 1), and *ATM* (N = 2) genes in 4 distinct patients.

Median age at diagnosis of first melanoma was 48.3 years (range 23-85) in the whole cohort, 51.1 (range 25-75) in patients with multiple melanomas, and 32.3 (range 25-44) in patients harboring *CDKN2A* PV, while the carrier of *MITF* PV presented his first melanoma at 51. All carriers of *CDKN2A* or *MITF* PV had a high nevus count (two had more than 100 nevi, the others between 50 and 100).

Among patients with ≥ 3 melanomas, the prevalence of *CDKN2A* PV was 18.2%, whereas it was 4.2% in patients with one or two melanomas.

All *CDKN2A* PV identified were missense and affected p16INK4A.

Clinical phenotypes and genetic test results of patients harboring a PV or a VUS are presented in Table 1. The general characteristics of the whole cohort are shown in Table S1.

Patient #7 carried the *CDKN2A* PV c.142C>A (p.Pro48Thr) (NM_000077.5:c.142C>A). He was diagnosed with five melanomas, the first at 28, with a total nevi number >100. He presented a positive family history of PC (paternal aunt) and possibly melanoma aggregation in the paternal lineage (his father developed a non-specified skin cancer in old age).

Patient #8 and patient #35 harbored the well-known c.301G>T (p.Gly101Trp) *CDKN2A* PV (NM_000077.5: c.301G>T), which is particularly frequent in Italy and France due to a founder effect [22]. Patient #8 developed four melanomas, the first at age 28. He had a total nevi number >50. Family history included PC (paternal uncle). He has a sister and a 32-year-old daughter, who refused to perform genetic counseling and testing. Patient #7 had four melanomas, the first at 25 years, with a total nevi number > 50. Family history included multiple melanomas (sister) and PC (maternal grandfather). Genetic counseling was extended to the mother, sister, and daughter, and at the time of this manuscript, the results were not available. Patient #35 developed two melanomas (at 44 and 56 years, respectively), his sister had at least four melanomas, and a paternal uncle had a melanoma at around 40 years. He had a total nevi number >50. The sister, the only sib of patient #35, underwent genetic testing and tested positive. Two out of his four children had genetic counseling and tested negative.

Patient #33 was found to carry the *MIFT* PV c.952G>A (p.Glu318Lys) (NM_000248.4:c.952G>A. This patient had two melanomas, the first at 51 years, with a total nevi number >100. His father developed a PC. Genetic testing was proposed to his brother, sister, and two children; however, to our knowledge, none was ultimately tested due to unwillingness.

Patient #26 tested positive for c.150+5del *CDKN2A* variant (NM_000077.5:c.150+5del), currently classified as VUS. This patient, diagnosed with melanoma at age 26, also had a family history of melanoma since his father presented two melanomas at 32 and 49. Therefore, a genetic analysis was also performed on the patient's father, who was a carrier of the same variant, to evaluate the segregation of this variant in the family.

Patient #24 had the c.272C>T (p.Pro91Leu) variant in the *MITF* gene (NM_001354604.2:c.272C>T) which is absent in ClinVar but reported in dbSNP database with the entry rs199832302. The variant is absent in GnomAD population database and is predicted to be neutral for the protein function by in silico predictors. It is important to highlight that this variant is absent in the transcript NM_000248.4 because of its upstream position from the start codon, and this aspect may limit its clinical interest in hereditary melanoma. However, this patient had a potentially suggestive family history in both parental branches: his father was diagnosed with clear cell kidney cancer aged 39 years, and his mother had thyroid cancer at 50 and melanoma at 59. The father renal cancer and the mother melanoma were confirmed through medical records. On the paternal side, an uncle presented with melanoma aged 55, and a second uncle with renal cancer aged 54. We could see the father patient in genetic consultation, and he accepted to undergo a genetic analysis that failed to identify the *MITF* c.272C>T (p.Pro91Leu), which was, in turn, of maternal origin.

Two VUS were detected in *ATM* gene in two distinct individuals.

The *ATM* c.8734A>G (p.Arg2912Gly) (NM_000051.4: c.8734A>G) variant found in patient #1 is currently classified as a VUS based on its presence at low frequency in GnomAD database in Non-Finnish European population (0.04%) with an homozygote carrier, altered protein function predicted by in silico tools and moderate aminoacid conservation score. The variant has been previously reported in ClinVar database as VUS.

The *ATM* c.3393G>A (p.Met1131Ile) (NM_000051.4: c.3393G>A) variant found in patient #5 is a missense variant classified as VUS based on the following: lack of effect on the protein function predicted by in silico tools , presence of previous classifications on ClinVar database, and extremely low variant frequency in Non-Finnish European population in GnomAD database (0.0004%).

No segregation analysis was performed in these two families.

Table 1. Clinical characteristics and genetic test results of patients harboring a pathogenic variant or a variant classified as variant of unknown clinical significance

Patient ID	N. of melanoma	N of melanoma in the family[a]	Age at diagnosis of the first melanoma	FH of PC[a]	Total nevus count[b]	Fitzpatrick skin type	Gene	Genetic change (HGVS nomenclature)	Protein change	Variant interpretation
#1	3	3	64	no	3	3	*ATM*	NM_000051.4:c.8734A>G	p.Arg2912Gly	VUS
#5	3	4	54	no	3	3	*ATM*	NM_000051.4:c.3393G>A	p.Met1131Ile	VUS
#7	5	7	28	yes	4	3	*CDKN2A*	NM_000077.5:c.142C>A	p.Pro48Thr	PV
#8	4	5	25	yes	3	3	*CDKN2A*	NM_000077.5:c.301G>T	p.Gly101Trp	PV
#24	1	2	39	no	3	1	*MITF*	NM_001354604.2:c.272C>T	p.Pro91Leu	VUS
#26	1	3	26	no	2	2	*CDKN2A*	NM_000077.5:c.150+5del		VUS
#33	2	2	51	yes	4	3	*MITF*	NM_000248.4:c.952G>A	p.Glu318Lys	PV
#35	2	7	44	no	3	3	*CDKN2A*	NM_000077.5:c.301G>T	p.Gly101Trp	PV

FH = family history; PC = pancreatic cancer; HGVS = Human Genome Variation Society; VUS = variant of unknown clinical significance; PV= pathogenic variant

[a] up to 2nd degree; [b] 1= <10, 2= 10-50, 3 =50-100, 4 =>100.

Predictors of Genetic Predisposition

We evaluated the patient phenotype (number of nevi and melanomas, age of first melanoma diagnosis) and family history of PC and melanoma among 1st- and 2nd-degree relatives as clinical predictors of the presence of a genetic predisposition to melanoma. As shown in Table 2, three out of the four patients harboring *CDKN2A* or *MITF* PV had a positive family history of PC (75%), whereas, among 31 patients without PV in tested genes, five had a 1st- or 2nd-degree relative with PC (16.1%) (P = 0.0302). In addition, having ≥ 50 nevi or at least four melanomas and observing ≥ 5 melanomas among patient and 1st- or 2nd-degree relatives were statistically significantly associated with PV in tested genes (P < 0.05), whereas a personal history of fewer than four melanomas and a young age (< 45 years) at first melanoma diagnosis did not predict a monogenic predisposition.

Conclusions

Given the stringent inclusion criteria employed, a higher percentage of patients carrying a PV in a melanoma-predisposing gene was expected in the presented series. However, the detection rate was only 11.4%.

The prevalence of *CDKN2A* PV was 8.6% in this study and was 18.2% considering patients with ≥ 3 melanomas and 4.2% in patients with 1 or 2 melanomas. This is remarkably lower than the 33% detection rate in the Italian population with FM reported in 2009 by the Italian Melanoma Intergroup study (25% in families with 2 melanomas and 46% in families with 3 melanomas) [13] and with a detection rate of 29.6% in the population with three or more melanomas reported in 2016 by the same group [6]. Interestingly, the authors selected patients according to Italian Society of Human Genetics recommendations, using less stringent clinical criteria (≥ 2 melanomas in a single patient or within the same family lineage regardless of age at diagnosis) than those employed in this study. These and other Italian studies [6,13] reported a *CDKN2A* mutation rate of 8.2% in patients with two melanomas, 17.6% in patients with three or more melanomas, 36.6% in patients with FM and two melanomas, 58.8% in patients with FM, and three or more melanomas, of 25% in families with two affected members, and of 46% in families with three affected members. In a study on multiple melanomas in the Veneto region, the detection rate of the *CDKN2A* PV for multiple melanomas was 12% [10].

Although these studies indicate a high prevalence of *CDKN2A* PV among FM cases in Italy, a recent Italian study showed a remarkably lower prevalence of PV (9.47%), considering either *CDKN2A* alone or the whole gene panel [14], close to the findings of our study. The authors suggested that, in the absence of a diagnosis of at least three primary melanomas, personal/family history of PC, and in patients with

Table 2. Detection rate according to patient cutaneous phenotype and family history among first- and second-degree relatives

Patient phenotype and FH	PV (any gene), N (%)	No PV, N (%)	Total, N (%)	Two-sided Fisher exact test
Negative family history of PC	1 (3.07%)	26 (96.30%)	27 (100%)	P = 0.0302
Positive family history of PC	3 (37.50%)	5 (62.50%)	8 (100%)	
Personal history of ≥ 50 nevi	4 (23.53%)	13 (76.47%)	17 (100%)	P = 0.0455
Personal history of < 50 nevi	0 (0%)	18 (100%)	18 (100%)	
< 45 years at first melanoma	3 (20.00%)	12 (80.00%)	15 (100%)	P = 0.2924
≥ 45 years at first melanoma	1 (5.00%)	19 (95.00%)	20 (100%)	
Personal history of ≥ 3 melanomas	2 (18.18%)	9 (81.82%)	11 (100%)	P = 0.5748
Personal history of < 3 melanomas	2 (8.33%)	22 (91.67%)	24 (100%)	
Personal history of ≥ 4 melanomas	2 (100%)	0 (0%)	2 (100%)	P = 0.0101
Personal history of < 4 melanomas	2 (6.06%)	31 (93.94%)	33 (100%)	
Personal history/family history of ≥ 4 melanomas	3 (27.27%)	8 (72.73%)	11 (100%)	P = 0.0819
Personal history/family history of < 4 melanomas	1 (4.17%)	23 (95.83%)	24 (100%)	
Personal history/family history ≥ 5 melanomas	3 (50.00%)	3 (50.00%)	6 (100%)	P = 0.0114
Personal history/family history < 5 melanomas	1 (3.45%)	28 (96.55%)	29 (100%)	

FH = family history; PC = pancreatic cancer; PV = pathogenic variant

melanoma diagnosed after 60, the benefit of genetic testing should be carefully evaluated due to the low detection rate. Our data support this position and emphasize that the inclusion criteria for hereditary melanoma genetic testing in Italy may need to be reconsidered. We suggest that the assessment for genetic counseling referral should be proposed with caution in cases involving sporadic multiple melanomas with only two occurrences and those with a late age of onset, especially for patients with I-II skin type and environmental risk factors. We speculate that the main burden of familial/multiple melanomas in our region may be linked to PV in untested moderate to low penetrance genes or in yet-to-be-discovered cancer predisposition genes, combined with environmental risk factors.

In line with the literature, all *CDKN2A* deleterious variants identified in our study were missense variants affecting p16INK4A [23]

The percentage of patients carrying *MITF* PV in this study was 2.9%, comparable to data from the literature [3,24]. *MITF* is a transcription factor involved in the homeostasis of melanocytes and other cell types. *MITF* c.952G>A (p.Glu318Lys) is a well-known PV conferring a moderate risk of developing melanoma. Moreover, multiple primary melanomas, increased nevus count, early-onset melanoma, and family history of melanoma have been reported in carriers of *MITF* c.952G>A (p.Glu318Lys) [24-26]. Although some authors have suggested a possible association between *MITF* c.952G>A (p.Glu318Lys) and PC and renal cancer, currently, there is not sufficient evidence to support clinical and radiological surveillance for cancers other than melanoma [14,25,27,28].

Focusing on VUS with an interesting potential of re-classification in "likely pathogenic", the c.150+5del variant falls in intron 1 of the *CDKN2A* gene and meets criteria for suspicion as it involves an intronic deletion, not at the splice donor/acceptor consensus site, but at a nearby site. This variant has been reported only once in global genetics databases. It has suspicious characteristics confirmed by the ACMG pathogenicity classification tool, which predicts this variant to be likely pathogenic. Together with the patient's personal and family history, these elements suggest a deleterious effect of the *CDKN2A* c.150+5del variant. However, in the absence of evidence of the impact of this variant on mRNA splicing, its classification remains pending.

We also reported in a distinct patient the *ATM* c.8734A>G (p.Arg2912Gly) variant, currently classified as a VUS. It is interesting to note that the Varsome ACMG classification tool classifies it as a "Likely Pathogenic" variant, based on disrupted protein function predicted by in silico tools, presence of functional and segregation studies that supports this prediction, involvement of a nucleotide, which falls in an hotspot sequence rich of PVs. However, its prevalence in GnomAD database, with one homozygote reported, the presence of this variant in control cases and its co-segregation with other PV strictly related to the observed phenotype in some studies suggests a more careful classification in VUS. No segregation studies have been performed for this variant in our series, but it is something to take in serious account for the future in order to investigate the potential re-classification.

In line with the findings of previous studies [14], we found a statistically significant association between the presence of PV in the panel of melanoma-predisposing genes tested and a family history of PC in up to second-degree relatives (P = 0.0302). Early diagnosis of PC seems possible thanks to ongoing research on surveillance in high-risk individuals with a hereditary predisposition. Surveillance with annual magnetic-resonance cholangiopancreatography or endoscopic ultrasound is recommended for individuals with known *CDKN2A* PV [15,16] starting from 40 or 30 in Italy [29,30]. The three carriers of *CDKN2A* PV in this study were referred to Centers dedicated to PC surveillance.

This study has limitations. First, it is a small study, with only 35 out of 46 eligible patients accepting to undergo genetic counseling and testing. Moreover, while the diagnoses of melanoma in the patients enrolled in this study were pathologically confirmed, it was not so for the diagnoses of PC or melanoma in family members and might not be accurate. Furthermore, the prevalence of *CDKN2A* and *ATM* PV is conditioned by the clinical classification of the *CDKN2A* c.150+5del variant and the *ATM* c.8734A>G (p.Arg2912Gly) variant that is, at present, unknown. Further functional studies, segregation, and clinical data of other family clusters could help define the impact of these gene variants.

Upon reviewing the obtained results, according to recent data from the Italian Melanoma Intergroup, we believe that clinical criteria to propose genetic counseling and testing to melanoma patients in Italy should be re-evaluated, adopting criteria in use in high-incidence melanoma countries. Moreover, despite its limitations, this series supports the genetic assessment for oncological hereditary predisposition in melanoma patients with a positive family history of PC up to the 2nd degree of kinship.

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