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### **Multiple primary melanomas (MPMs) and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian Melanoma**

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# Multiple primary melanoma and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian Melanoma Intergroup

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## **Abstract for JAAD:**

### **Background:**

It is currently accepted that multiple primary melanoma (MPM), in concert with a positive family history (FH), is a good predictor of germline mutations in the cyclin dependent inhibitor 2a gene (*CDKN2A*). In particular, a useful rule of thumb regarding the presence of either two or three or more cancer events (i.e., melanoma and pancreatic cancer) in low or high melanoma incidence populations, respectively, has been established to select patients (pts) for genetic referral. However, studies on low melanoma incidence populations could be biased by founder mutations.

**Objective:** Aim of this Italian study was to determine the *CDKN2A/CDK4/MITF* mutation rate among MPM patients on a national basis in order to appropriately direct genetic counseling, regardless of FH.

**Methods:** 587 MPM pts, and an equal number of single primary melanomas (SPM) and controls were consecutively enrolled at the participating centers and tested for *CDKN2A*, *CDK4* and *MITF* genes.

**Results:** Germline mutations in *CDKN2A* were found in 19% of MPM pts vs 4.4% of SPM pts. In MPM familial cases the mutation rate varied from 36.6% in pts with two melanomas to 58.8% in pts with three or more melanomas, while in sporadic MPM cases the mutation rate varied from about 8.2% in pts with two melanomas to 17.6% in pts with three or more melanomas. The *MITF* E318K mutation accounted for 3% of MPM cases altogether.

**Limitations:** The study was hospital-based and not population-based. Rare novel susceptibility genes were not tested.

**Conclusion:** Italian patients who develop at least two melanomas, even in situ, should be referred for genetic counseling even in the absence of FH

### **Capsule summary:**

- *CDKN2A* is the main candidate gene for germline testing in melanoma families and MPM patients. In Italy mutation prevalence is influenced by founders
- Despite regional differences, Italian *CDKN2A* mutation rate is about 10% even in MPM patients with only two melanomas, including in situ melanomas
- MPM patients from Italy warrant genetic testing regardless of FH

### **Keywords**

Melanoma, Pancreatic cancer, CDKN2A, CDK4, MITF, genetic assessment, family history, mutation

### **Abbreviations**

Cutaneous melanoma CM

Family history FH

Multiple primary melanoma MPM

Single primary melanoma SPM

Pancreatic cancer PC

Age at diagnosis AAD

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### **BACKGROUND**

Cutaneous melanoma (CM) is an often fatal form of skin cancer whose incidence is on the rise in many Caucasian populations<sup>1–3</sup>. The etiology of CM involves host characteristics and environmental risk factors, however the main risk factor for CM is positive family history (FH) for the disease, presenting with multiple melanoma events in family members<sup>4,5</sup>. Germline mutations in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) (*INK4a*) are reported in 5 to 40% of hereditary cases of melanoma, representing 5-10% of all melanoma cases, thus making it the most significant high-risk melanoma susceptibility gene identified to date. Germline mutations, in its binding partner cyclin-dependent kinase 4 (*CDK4*), are very rare<sup>6–11</sup>. The differences in the background incidence of melanoma and penetrance of *CDKN2A* mutations among countries are such that there is no single guideline for genetic testing that could be applied worldwide<sup>12,13</sup>. In the context of an agreement within the International Melanoma Consortium (GenoMEL), Leachman et al. proposed that only two cancer events, including pancreatic cancer (PC), either in the patient or in a family member are criteria enough to best identify which patients would benefit most from genetic testing in low melanoma incidence areas<sup>13</sup>. Italy was included among these areas on the basis of mutation prevalence data from the Ligurian population<sup>12,14–18</sup> where *CDKN2A* founder mutations were prevalent in up to 40% of melanoma families in concert with a strong association with pancreatic cancer<sup>19,20</sup>. The first Italian cooperative study based on the SIGU (Italian Society of Human Genetics) protocol for melanoma families undergoing genetic counseling found that 33% of the families overall and 25% of those with only two affected members carried *CDKN2A* mutations<sup>21,22</sup>. A significant increase in the frequency of mutations was observed in patients whose family members also had multiple primary melanomas (MPM). In addition, a

Ligurian hospital-based study of single primary melanoma (SPM) and MPM found that the frequency of *CDKN2A* mutations in MPM cases was 32.6%, and that from 8 to 15% of MPM without a FH of CM harbored a *CDKN2A* mutation<sup>23</sup>. Recent estimates of the prevalence and predictors of *CDKN2A* mutations for people from regions with widely differing latitudes and melanoma incidence (UK, Australia, Spain) showed that there is a low probability (<2%) of detecting a germline *CDKN2A* mutation in people with melanoma except for those with a strong FH of melanoma (2 affected relatives, 25%), three or more primary melanomas (29%) or more than one primary melanoma who also have other affected relatives (27%). For instance, in Spain, mutation prevalence was 8.7% for MPM without a melanoma FH, 7.1% for subjects with a melanoma FH but only one primary and 17.4% for patients with MPM and a melanoma FH. A similar pattern was seen for UK patients (7.5%, 5.7% and 45.4%)<sup>24</sup>. In France, another low melanoma incidence country, the frequency of *CDKN2A* mutations in families with 2 melanoma patients was 13%, but this percentage rose to 22% when the median age at melanoma diagnosis was younger than 50 years and to 29% when there was 1 or more subjects with MPM<sup>25,26</sup>. Recently, a melanoma predisposing mutation was identified in Microphthalmia-associated transcription factor (*MITF*), and a Ligurian study supported the hypothesis of *MITF* as a medium-penetrance melanoma susceptibility gene<sup>27–30</sup>. Several novel melanoma susceptibility genes (*POT1*, *BAP1*, *TERT*, *ACD*, *TERF2IP*) have also been identified, but their geographical prevalence and penetrance has yet to be established<sup>31–36</sup>. Based on these observations, the aim of our study was to carry out a nationwide evaluation of the mutation rate of melanoma susceptibility genes *CDKN2A*, *CDK4*, *MITF* and associated features in MPM patients to establish whether even in the absence of FH, MPM may be added as a single criterion to update the national recommendations for genetic testing for hereditary melanoma.

## METHODS

### Case selection

The study was performed on 587 MPM and SPM patients consecutively enrolled during their follow-up between 2010 and 2012 and on control subjects, (i.e., friends, spouses, colleagues, blood donors), in order to evaluate genetic variants that had not previously been described, for a total of 1,749 samples. The participating Italian Melanoma Intergroup (IMI) centers included: Genoa (University and IRCCS AOU San Martino IST), Padoa (Veneto Institute of Oncology -IOV), Milan (Fondazione IRCCS Istituto Nazionale Tumori and European Institute of Oncology), Bergamo (Ospedali Riuniti), Florence (University and Santa Maria Annunziata Hospital), Turin (University and Gradenigo Hospital), Naples (Pascale foundation), Sassari (National Center of Research-CNR), Varese (Ospedale di Circolo University of Insubria). The number of melanomas, age at diagnosis (AAD), diagnostic pathological data, cancer family history and phenotyping were recorded for each patient through a standardized questionnaire. Due to the known association with mutations in *CDKN2A*, confirmation was requested for reported diagnoses of PC in the family, at least by medical records when death certificates or pathology reports were not available. Confirmation was obtained for about 50% of PC cases while the remaining ones were reported by family members. All of the study patients were enrolled between 2010 and 2013 during their follow-up. Only SPM patients who had been diagnosed at least 3 years prior to the beginning of our study were recruited due to the increased risk of a second melanoma during the two years after the diagnosis<sup>37,38</sup>.

Each participating center recruited at least 20 patients with MPM, 20 patients with SPM and a corresponding number of controls. Written informed consent was obtained from all participants according to ethics committee approved protocols.

### Molecular analyses

The same standard protocol for testing was followed at all the centers that performed molecular analyses. Samples from the European Institute of Oncology as well as from the centers of Turin, Varese and Bergamo were sent to Genoa for testing, whereas samples from the remaining centers were tested locally. In order to perform a quality check, 10% of the 282 samples tested by other laboratories were selected randomly by the coordinating center of Genoa and blindly analyzed. No discrepancies were detected among the molecular results. Genomic DNA was extracted from peripheral blood using standard methods. The *CDKN2A* coding region, including splice junctions, the 5'UTR, the intronic sequence known to contain the IVS2-105A/G mutation and exon 1 $\beta$  was entirely sequenced, as were *CDK4* exon 2 (no causal mutations have ever been identified outside this exon) and *MITF* exon 10 as previously described<sup>14,15,22,29,39–41</sup>. Multiplex ligation-dependent probe amplification (MLPA) analysis was also performed to exclude *CDKN2A* large deletions or duplications in a subset of 40 samples with MPM and FH with sufficient DNA of adequate quality, using the SALSA MLPA kit ME024 9p21 *CDKN2A*/2B (MRC-Holland) as previously described<sup>42</sup>. The type of *CDKN2A* (exons 1 $\alpha$ , 2, and 3), *ARF* (exon1 $\beta$ ), *CDK4* (exon 2) or *MITF* mutation was recorded for each mutation-positive patient. Mutation nomenclature conforms to the complementary DNA numbering as recommended by the Human Genome Variation Society; mutation numbering referred to NM\_000077.3. For novel variants, prediction of any deleterious effects on protein was performed by bioinformatic criteria based on SIFT (<http://sift.jcvi.org/>), Polyphen (<http://genetics.bwh.harvard.edu/pph/>), pMut (<http://mmb2.pcb.ub.es:8080/PMut/>) or SpliceView([http://bioinfo4.itb.cnr.it/~webgene/wwwspliceview\\_ex.html](http://bioinfo4.itb.cnr.it/~webgene/wwwspliceview_ex.html)). These variants were classified as unknown variants (UV) and carriers were excluded from the overall calculation of mutation rate when no conclusive evidence of pathogenicity was obtained.

### Statistical analyses

Statistical correlation between *CDKN2A* mutations and clinical or pathological variables was performed by  $\chi^2$  tests. Comparisons between categorical variables were performed with  $\chi^2$  tests and Fisher corrections where required. All analyses were two tailed and p values of less than .05 were considered statistically significant. Results are reported as odds ratio (OR) and 95% confidence intervals (CI).

## RESULTS

A total of 112/587 (19%) of MPM patients and 26/587 of SPM (4.4%) harbored *CDKN2A* mutations, regardless of FH (Table 1). No genomic alterations were detected by MLPA in a selection of 40 out of 180 *CDKN2A* mutation-negative MPM samples with a higher risk of being carriers of genetic alterations (familial cases or MPM cases with 3 or more melanomas), confirming results that had previously been described in the Italian population<sup>42</sup>. Only one SPM patient showed a mutation in *CDK4* (c.70C>T, p.R24C)<sup>11</sup>(Table 2). No mutation was found among controls. As expected, the most common mutation was the founder G101W, which was detected in 56% of the cases (Figure 1). The second most frequent mutation was E27X followed by P48T and R24P (7% and 5% respectively) mutations, previously described as founder or recurrent mutations<sup>15,17,22,40,43</sup>. Several novel variants were observed, i.e., S56R and F90S and c.280\_282insAG c.151-18T>C&c.151-13T>C in *CDKN2A* and c.193+54C>T in p14arf (Table 2). The S56R and F90S variants we found in the coding regions were classified as pathogenetic based on public *in silico* prediction tools (Polyphen, Sift and pMUT) and on their absence in healthy population controls. The c.280\_282insAG also determines a stop codon downstream. No conclusions could be drawn for the *CDKN2A* c.151-18T>C & c.151-13T>C and the p14arf c.193+54C>T as these variants were predicted to have no effect on mRNA processing using the



Splice View prediction tool, but were not found in healthy controls. As for the *CDKN2A* c.150+37G>C, there is no conclusive evidence-regarding pathogenicity, even if a causal role can not be excluded due to its absence in the control populations and the correlated alteration of the *CDKN2A* isoform 3<sup>44</sup>. These variants were classified as UV. The other mutations were previously described with evidence of pathogenicity<sup>45–48</sup>

The highest mutation rate in MPM cases was found in the northern regions of Italy, particularly in Liguria and Lombardia, followed by Veneto (35%, 24% and 12% respectively), while the percentage decreased in central regions, though remaining above or near 10% (Figure 2). When FH was taken into account, we observed that the prevalence of *CDKN2A* mutations in MPM patients was as high as 44.4%. Interestingly, despite regional differences, a considerable proportion of the MPM patients without FH (10.8%) harbored *CDKN2A* mutations (Table 1). The frequency of mutations increased significantly as the number of melanomas rose, going from 14.6% in subjects with two melanomas to 29.6% in those with three or more melanomas ( $p < 0.0001$ ) (Table 3). In familial MPM cases the mutation rate varied from 36.6% in pts with two melanomas to 58.8% in pts with three or more melanomas ( $p = 0.0139$ ), while in sporadic MPM cases the difference in mutation rate varied from 8.2% in patients with two melanomas to 17.6% in patients with three or more melanomas ( $p = 0.0062$ ).

Since *in situ* melanomas accounted for about 22% of our MPM cohort, we wondered whether including these lesions could have modified or biased the observed mutation rates. We performed the analyses by excluding cases with *in situ* melanomas in order to quantify their influence on the total mutation rate. We did not find any significant differences in the mutation rate between the categories we studied, or among all MPM ( $p = 0.5594$ ) or familial ( $p = 0.7249$ ) or sporadic MPM ( $p = 0.593$ ), while excluding *in situ* melanomas from our cohort would have implied the loss of a very significant proportion of mutation carriers.

The overall *CDKN2A/CDK4* mutation rate in SPM was 4.4% (26/587) vs 19% of MPM cases and while 24.6% of familial SPM were *CDKN2A*-positive (15/61), only 2.1% of sporadic SPM were mutation carriers (11/526) vs 8.2% of sporadic MPM pts with only 2 melanomas ( $p < 0.0001$ ) (Table 1). The sporadic SPM mutation carriers showed a slightly significant association with the presence of PC, both in the patient and among 1st or 2nd degree relatives ( $p = 0.0023$ ) (Table 4). The cases with an insufficient degree of confirmation of cancer FH were excluded. The same comparison among MPM patients with or without PC resulted in a highly significant difference ( $p = 0.0002$ ). The median AAD of MPM patients was 45 years (range 15-91) and it was significantly different ( $p < 0.0001$ ) in *CDKN2A*-positive patients (39 years in familial cases vs 38 years in sporadic cases,  $p = 0.7280$ ) compared to patients with no mutations (44 years in familial cases vs 48 years in sporadic cases,  $p = 0.0443$ ).

The median AAD of SPM pts was 49 years (range 15-89). The difference between mutation carriers and wild type (WT) pts was not statistically significant (37 years in familial cases vs 46 years in sporadic cases,  $p = 0.0950$ ). Moreover, even the difference between sporadic mutation carriers and WT SPM was not significant (46 years vs 48 years,  $p = 0.8038$ ), and the AAD of sporadic WT SPM and MPM pts was the same.

In an effort to reach national agreement regarding the implementation of *MITF* analysis in the routine diagnostic work up for melanoma susceptibility, we evaluated the prevalence of *MITF* mutations in those melanoma patients for whom *MITF* analysis had been performed after ruling out *CDKN2A/CDK4* mutations. Two cases (one sporadic MPM and one familial SPM) carrying the mutation in both *MITF* and *CDKN2A* were also excluded from the analysis. The *MITF* E318K mutation rate in *CDKN2A/CDK4* negative MPM was 3.2 %, while in SPM cases it was 0.7%. A comparison between MPM cases vs SPM cases showed a stronger association of *MITF* with multiple events in both familial and sporadic cases (Table 5).

## DISCUSSION

In 2002, despite considering genetic testing premature, GenoMEL recognized that in countries such as Italy, where melanoma incidence is low but founder mutations are common, genetic testing can improve adherence to surveillance programs among mutation carriers<sup>6</sup>. Indeed, Italy was one of the first countries where clinical genetic testing for familial melanoma was offered in genetics services. In 2004, SIGU drafted its recommendations, which have since become the protocol adopted by Italian genetics services for access to genetic counseling<sup>21</sup>. The 2009 cooperative study showed that clinical genetic testing for melanoma in Italian families with just two affected members is justified in terms of the likelihood of identifying a *CDKN2A* mutation<sup>22</sup>. Moreover, the same study suggested that the presence of MPM cases increases the likelihood of identifying mutation carriers, and a regional, hospital-based study showed that a significant percentage of MPM pts were *CDKN2A* mutated regardless of FH<sup>23</sup>. This multicenter study performed on a national basis in the context of the Italian Melanoma Intergroup confirms that despite regional differences due to the presence of founder-mutations (Figure 2), the development of MPM even in the absence of melanoma FH can be considered a new single criterion for referral to genetic testing on a national basis. Although in terms of the likelihood of being a mutation carrier the development of three or more melanomas is not comparable to also having a melanoma FH, the percentage of sporadic MPM mutation carriers is still about 10% on a national basis, considering areas where founder mutations are not prevalent and consequently *CDKN2A* mutation rates are lower. When FH is positive, the presence of MPM cases is confirmed as being a strong mutation-predictive parameter. Notwithstanding, although the results of SPM analysis confirm that familial cases, thus even with only two affected family members, show a high percentage of mutation carriers, the rate is nonetheless below 5% when FH is not present. Currently, SIGU recommendations do not include the presence of PC in the proband or in relatives among the criteria for access to genetic counseling given that data on PC risk in melanoma families are available only for the Italian region of Liguria and that there is no national agreement for a protocol that could be offered to individuals at high-risk of PC<sup>16,19,20</sup>. Leachman et al. proposed that one melanoma and a case of pancreatic cancer in a family suffice to best identify which patients would benefit most from genetic testing in low melanoma incidence areas such as Italy<sup>13</sup>. The result we found in SPM with only one PC case in the family or in the same proband support the validity of the internationally proposed criteria for low melanoma incidence areas, including PC as the second cancer event other than melanoma in the Italian population at a national level. Therefore, the presence of PC in the proband or in a family member should be taken into account when deciding on referral. However, since further refinement of the criteria for identifying patients at high risk for PC on the basis of genetic data rather than life-style factors is still needed, as is a nationally approved surveillance protocol, we suggest that in the meantime the association between *CDKN2A* mutations and PC should be managed carefully by the referring clinicians and genetic counselors.

In this study we decided to combine invasive and *in situ* melanomas in the same analyses. *In situ* melanomas were often not counted as melanomas for the purposes of genetic studies or risk assessment, even if patients with *in situ* melanomas have a higher risk of developing invasive melanoma and they are enrolled in screening protocols<sup>49</sup>. Nevertheless, a recent northern European study proved that *in situ* melanomas confer a familial risk equal to that of invasive melanomas, thus also suggesting that even *in situ* MPM cases-have to be considered among the selection criteria for genetic assessment of patients with familial melanoma<sup>50</sup>. *In situ* melanomas actually accounted for about 22% of our MPM cohort and our analysis also found that the extent to which *in situ* melanomas and invasive melanomas contribute to the mutation rate is comparable.



Some Italian genetics centers currently analyze MITF in melanoma cases, but only for research purposes. In the view that the MITF gene can be routinely tested for diagnostic purposes, molecular analysis shows that this novel susceptibility gene is responsible for sporadic MPM susceptibility in about 3% of cases. Due to the strong correlation with multiple melanoma events, we suggest that diagnostic testing in MPM cases could be improved by molecular analysis of the MITF gene.

Among the minor observations, although younger age of onset is a feature of *CDKN2A* mutations, in the absence of FH the selection of patients based on young age at melanoma diagnosis alone does not result in a sufficiently high likelihood of finding a mutation to warrant referral<sup>13</sup>. Nevertheless, we have observed an alarming trend towards lower AAD in *CDKN2A*-negative subjects, which is currently approaching the AAD of mutation carriers.

One of the limits of the study was that despite being a multicenter study it is not population-based and not all Italian regions were represented, (i.e., the central eastern coast and southern Italy). However, most of the centers participating into the Italian Melanoma Intergroup perform genetic counseling and testing as well as dermatological follow-up visits on many patients coming from these areas, especially from southern Italy. As we have pointed out, founder mutations are common among hereditary melanoma cases in some Italian regions, but this high prevalence in a defined area can not imply a national predictive value<sup>15,17,43,51,52</sup>. In general, the possibility that founder effects influence the overall picture of *CDKN2A* mutation prevalence in our population should be carefully considered. Other Italian regions showed a lower *CDKN2A/CDK4* mutation rate among melanoma families and in sporadic melanoma cases<sup>41,52-54</sup>. For instance, a rare founder mutation in *POT1* was recently identified in five unrelated melanoma families from Romagna, Italy<sup>31</sup>. Rare mutations in novel melanoma susceptibility genes (*BAP1*, *TERT promoter*, *ACD*, *TERF2IP*) have been identified in some melanoma families<sup>33-36</sup>. In this study these novel susceptibility genes were not tested. However, mutations in such genes overall occur in less than 10% of melanoma families with a yet unknown genetic prevalence in the studied populations. In the view of implementing a more widespread use of NGS methods, e.g. gene panels in clinical genetic testing, further population studies are needed to establish the mutation prevalence and penetrance of these genes in different countries. A melanoma susceptibility gene panel could be integrated with medium and low penetrance variants, e.g. *MITF*, *MC1R* and pigmentation genes, to gain an increasingly better definition of the personalized risk for each patient, one of the major challenges in managing the complex genetic picture of melanoma.

In conclusion, our study shows that despite regional differences, Italian patients presenting with only two melanomas and no FH warrant clinical genetic testing and that *CDKN2A* mutations confer an increased risk of melanoma independently of its natural history due to lack of a significant difference between in situ and invasive melanomas.

**Table 1. CDKN2A Mutation rates in MPM and SPM by family history**

	ALL		FAMILIAL		SPORADIC	
	MPM	SPM	MPM	SPM	MPM	SPM
<b>Mut</b>	112	26	64	15	48	11
<b>WT</b>	475	561	80	46	395	515
<b>TOT</b>	587	587	144	61	443	526
<b>Mut%</b>	19	4.4	44.4	24.6	10.8	2.1

**A comparison of CDKN2A mutation rates between Multiple Primary Melanoma cases (MPM) and Single Primary Melanoma cases (SPM): all together; only familial cases; only sporadic cases. Mut=mutation carriers. WT=wild-type.**

**Table 2. CDKN2A/CDK4 mutations in MPM and SPM**

<b>MPM</b>	<b>SPM</b>	<b>CDKN2A ink4</b>	<b>p16/p14 AA change</b>
62	16	c.301G>T	p.G101W/p.R115L
7	-	c.142C > A	p.P48T
6	1	c.71G>C	p.R24P
4	5	c.79G>T	p.E27X
3	-	c.301G>C	p.G101R/p.R115P
3	-	c.339G>T	p.A127S
2	-	c.379G>C	p.L113L/p.P114S
2	-	c.251A>T	p.D84V
2	-	c.68G>A	p.G23D
2	-	c.66_67GG>AA	p.G23S
2	-	c.194T>C	p.L65P
1	-	c.202_203GC >TT	p.A68L/p.R82L
1	-	c.263A>G	p.E88G
1	-	c.67G>C	p.G23R
1	-	c.449G>T	p.G150V
1	1	c.294C>T	p.H98H/p.P113S
1	-	c.149A>G	p.Q50R
1	-	c.148C>T	p.Q50X
1	-	c.172C>T	p.R58X
1	-	c.296G>C	p.R99P
1	-	c.167G>T	p.S56I/p.Q70H
1	-	<u>c.168 C&gt;A</u>	<u>p.S56R/p.R71S</u>
1	-	c.229A>G	p.T77A/p.H91R
1	-	c.280_282insAG	
1	-	c.-25C>T & c.-180G>A	
1	-	c.-34G>T	
1	-	c.58delG	c.58delG
-	1	<u>c.269T&gt;C</u>	<u>p.F90S</u>
-	1	c.281T>C	p.L94P
1	2	<i>c.150+37G&gt;C</i>	
1	-	<u><i>c.151-18T&gt;C &amp; c.151-13T&gt;C</i></u>	
	-	<b>CDKN2A (exon 1β)</b>	
1	-	g.193+1 G>A	
1	-	<u><i>c.193+54C&gt;T</i></u>	
-	-	<b>CDK4</b>	<b>CDK4 AA change</b>
-	1	c.70C>T	R24C

**Novel germline variants are underlined . Variants with unknown significance (UV) are in italics.**

**Table 3. CDKN2A Mutation rates in MPM and number of melanomas in correlation with the number of melanoma occurrences in a single patient**

MPM	ALL		FAMILIAL		SPORADIC	
	2	≥3	2	≥3	2	≥3
Mut	60	52	34	30	26	22
WT	351	124	59	21	292	103
TOT	411	176	93	51	318	125
Mut%	14.6	29.6	36.6	58.8	8.2	17.6

**Table 4. Association of CDKN2A mutations with pancreatic cancer in SPM and MPM patients**

	SPM		MPM	
	PC	No PC	PC	No PC
Mut	3	9	11	101
WT	11	378	21	441
TOT	14	387	37	537
OR	8.84		2.29	
95% C.I.	(2.18-35.85)		(1.06-4.89)	
p	0.0023		0.0331	

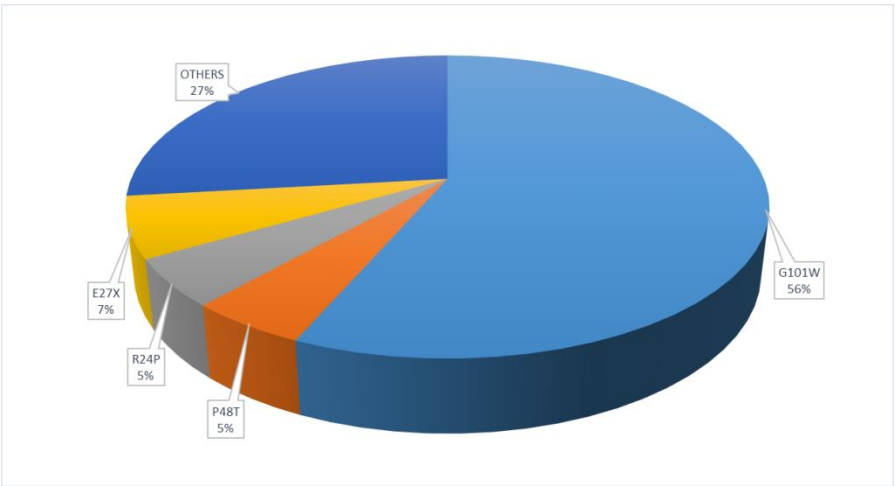
**Correlation between the presence of PC in a patient and/or in the family and the presence of CDKN2A mutations in SPM and MPM cases.**

**Table 5. Prevalence of the MITF E318K mutation in MPM and SPM on a national basis**

MITFE318K	ALL		FAMILIAL		SPORADIC	
	MPM	SPM	MPM	SPM	MPM	SPM
Mut	12	3	1	0	11	3
WT	366	411	69	39	297	372
TOT	378	414	70	39	308	375
Mut %	3.2	0.7	1.4	0	3.6	0.8
OR	4.49		1.71		4.59	
95% C.I.	(1.26-16.04)		(0.08-42.86)		(1.27-16.61)	
p	0.0207		0.7457		0.021	

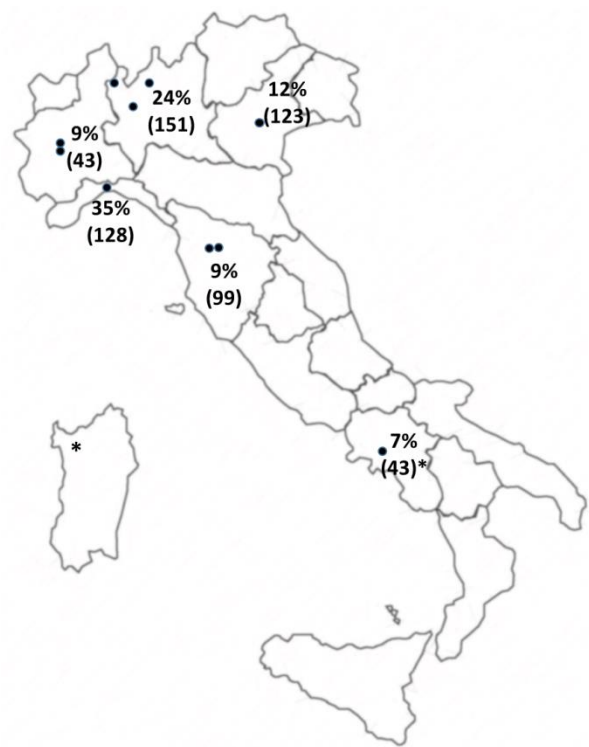
**A comparison of MITF mutation rates between Multiple Primary Melanoma cases (MPM) and Single Primary Melanoma cases (SPM): all together, only familial cases and only sporadic cases. Mut=mutation carriers WT=wild-type**

**Figure 1 *CDKN2A* mutation distribution**



*Frequency of mutation-positive patients is indicated after each mutation name.*

**Figure 2 *CDKN2A* mutation regional distribution**



Percentage of germline *CDKN2A* mutations in MPM patients and number of MPM patients is indicated in brackets for each region . Dots or asterisks show the geographic location of participating centers. Asterisks were used for patients from Naples and Cagliari which are referred to the same center for testing.

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