ORIGINAL ARTICLE

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

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Background: Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) is the major high-risk susceptibility gene for melanoma.

Objective: We sought to evaluate the effect of *CDKN2A* mutations in Spanish patients with a high risk of developing melanoma and the association with clinical and family history features.

Methods: A cross-sectional study design was used to analyze the *CDKN2A* impact in 702 Spanish patients with a high risk of developing melanoma.

Results: The *CDKN2A* mutation prevalence was 8.5% in patients with sporadic multiple primary melanoma and 14.1% in familial melanoma. Number of cases in the family, number of primary melanomas, and age of onset were associated with the presence of *CDKN2A* mutation. Having a *CDKN2A* mutation in the family increased the prevalence of other cancers (prevalence ratio [PR] 2.99, $P = .012$) and prevalence of pancreatic (PR 2.97, $P = .006$), lung (PR 3.04, $P < .001$), and breast (PR 2.19, $P = .018$) cancers but not nephrourologic or colon cancer.

Limitations: Smoking status was not assessed in the individuals with lung cancer.

Conclusions: Melanoma-prone families with mutations in *CDKN2A* have an increased prevalence of a broad spectrum of cancers including lung, pancreatic, and breast cancer. This information should be included in genetic counseling and cancer prevention programs for *CDKN2A* mutation carriers. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2014.06.036.)

Key words: breast cancer; *CDKN2A*; genetic counseling; lung cancer; melanoma; pancreatic cancer; prevention; risk; smoking.

Melanoma is a complex and heterogeneous disease, involving environmental, phenotypic, and genetic risk factors. Sunlight is the major environmental risk factor for melanoma[1] and phenotypic characteristics such as skin, eye, and hair color and the number of common and atypical cutaneous nevi are melanoma risk factors.[2,3]

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Conflicts of interest: None declared.

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Approximately 5% to 10% of melanoma cases occur in a familial context.[4] To date, 2 high-penetrance genes have been implicated in melanoma susceptibility: cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and cyclin-dependent kinase 4 (*CDK4*). Germline mutations in the *CDKN2A* gene have been observed in 20% to 40% of melanoma-prone families.[5] This gene codes for the tumor suppressor proteins p16 cyclin-dependent kinase 4 inhibitor A (p16INK4) and p14 alternative reading frame (p14ARF), both involved in cell cycle inhibition through different pathways.[6] Germline mutations in *CDK4*, an oncogene encoding one of the binding partners of p16INK4, are restricted to a few melanoma families.[7]

CAPSULE SUMMARY

- In addition to melanoma, other cancers have been observed in cyclin-dependent kinase inhibitor 2A—mutated melanoma-prone families.
- The study has identified an increased prevalence of pancreatic, breast, and lung cancer in melanoma-prone families carrying cyclin-dependent kinase inhibitor 2A mutations.
- These findings may improve the prevention strategies indicated for cyclin-dependent kinase inhibitor 2A mutation carriers.

In addition to melanoma, other cancers have been observed in *CDKN2A*-mutated melanoma-prone families and several studies have shown an increased risk of pancreatic cancer among these families.[8,9] In families carrying mutations, the relative risk of developing pancreatic cancer was 7.4 or 14.8 in studies from the United States[10] and Italy,[11] respectively. Furthermore, an increased risk of breast cancer has been observed in *CDKN2A*-mutated pedigrees.[12] In the first Spanish *CDKN2A*-mutated family, an increased risk for lung and breast cancers was also suggested.[13]

The identification of high-risk penetrance melanoma genes, which in turn are related to phenotypical characteristics of patients and number of cases within families, has allowed us to recommend genetic counseling for familial melanoma. Genetic counseling is a nondirected process offered to families to help them understand the meaning of the disease, the meaning of genetic susceptibility, the patterns of inheritance, the option of genetic testing, the understanding of all the possible results, and primary and secondary prevention.[14] To date, in low melanoma incidence populations the inclusion criteria for genetic testing in melanoma are: 2 (synchronous or metachronous) primary melanomas in an individual or families with at least 2 melanoma cases in first- or second-degree relatives.[15]

Also, the presence of pancreatic cancers among first- or second-degree relatives of the patients with melanoma has been considered as a selection criterion for genetic counseling.[15] Thus, identification of other malignancies related to *CDKN2A*-mutated families may be useful to refine the selection criteria and to improve preventive strategies. The aim of this study is to investigate which clinical and familial history features are associated with the presence of germline *CDKN2A* mutations in Spanish patients with a high risk of developing melanoma.

METHODS

Patients

A cross-sectional study design was used to analyze the *CDKN2A* impact in patients with a high risk of developing melanoma. Overall, 702 patients with melanoma were included in the study: 236 patients with sporadic multiple primary melanoma (SMP), and 466 patients with familial melanoma belonging to 330 high-risk melanoma-prone families with at least 2 melanoma cases (269 families with 2 melanoma cases, 47 families with 3 melanoma cases, 11 families with 4 melanoma cases, and 3 families with 5 melanoma cases). The patients included in the study were consecutively recruited from January 1992 to June 2013.

According to the number of tumors and the presence of other cases in the family, the set of patients included: patients with sporadic melanoma and multiple primaries (SMP, n = 236); melanoma patients with multiple primary melanoma (MPM) and familial history of melanoma (familial MPM [FMP], n = 115); and patients with melanoma and a single primary melanoma and family history of melanoma (n = 351).

The variables included in the analyses were age of onset, number of primary melanomas, number of melanoma cases within the family, and the presence of other cancers in first- and second-degree relatives of the patients with melanoma. We evaluated, specifically, whether first- and second-degree relatives developed pancreatic, colon, lung, nephrourologic (including kidney, bladder, or prostate), or breast cancers. We focused on those cancer types previously related to *CDKN2A* germline mutations such as pancreatic cancer and we have also included the most common cancers in Catalonia, Spain (colon, lung, breast, prostate, and bladder).[16] The cancer history was obtained from personal

Abbreviations used:

CDK4:	cyclin-dependent kinase 4
CDKN2A:	cyclin-dependent kinase inhibitor 2A
FMP:	familial multiple primary melanoma
MPM:	multiple primary melanoma
PR:	prevalence ratio
SMP:	sporadic multiple primary melanoma

interviews conducted the day of the melanoma diagnosis or during the follow-up.

Age of onset information was available for more than 90% of the patients and family history of other cancers was available in 90% of melanoma-prone families and 80% of SMP. A questionnaire about smoking habits was obtained from 172 individuals belonging to 75 melanoma-prone families. The set included 54 (31.4%) *CDKN2A* mutation carriers and 118 (68.6%) wild-type individuals. The smoking habits were classified as: never, former, or current smokers. The age of daily smoking onset and the number of cigarettes per day were also recorded for current and former smokers. Smoking habits from the general population from Catalonia were collected from the National Statistics Institute of Spain for the period July 2011 to June 2012.

All patients were selected from the melanoma unit database from the Hospital Clinic of Barcelona. The study was approved by the ethical committee of the Hospital Clinic of Barcelona. The patients gave their written, informed consent.

Mutational analysis

Genomic DNA was obtained from peripheral lymphocytes of the patients with melanoma included in the study according to the salting-out method.[17] The *CDKN2A* locus (exon 1α, 1β, 2, and 3) and *CDK4* exon 2 were amplified by polymerase chain reaction as previously described.[13,18,19]

Statistical analysis

The 2-sided χ^2 or Fisher exact test, as appropriate, was used to test for statistical significance in proportion comparison. Continuous variables, such as the age at diagnosis, were tested using the analysis of variance test. Analysis of familial history of other cancers was carried out by classifying the pedigrees as to absence or presence of a given type of cancer, and calculating the prevalence ratio (PR) and its 95% confidence intervals.

The functional effect of each genetic variant detected in *CDKN2A* was evaluated *in silico* using PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/).[20] We excluded 1 SMP and 3 families because they carried mutations that did not alter the protein function. Furthermore, we excluded 1 other SMP and 1 family that carried mutations that cause an amino acid change in the protein because in silico analyses predicted them to be benign and they were not present in all the affected members in the family. The COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) was used to evaluate whether previously unreported germinal mutations were observed at the somatic level.

Bonferroni correction was applied in multiple analyses and an adjusted *P* value was obtained multiplying the test *P* value for the number of comparisons performed. The result was considered statistically significant if *P* value or adjusted *P* value, as appropriate, was less than .05. Software (SPSS 17.0, IBM Corp, Armonk, NY) was used for the statistical analyses.

RESULTS

CDKN2A and *CDK4* were tested in 702 patients with melanoma: 236 patients with SMP and 466 with familial melanoma belonging to 330 high-risk melanoma-prone families with at least 2 melanoma cases. Overall, 32 germline *CDKN2A* mutations were identified: 18 previously described mutations,[13,19,21-31] 9 mutations previously observed at the somatic level,[32,33] and 5 novel unreported mutations (data available on request). *CDK4* mutations were not observed.

Several sporadic cases or families were removed from the study based on different criteria. A SMP case carrying a predicted benign mutation that has not been previously described (p.G32R located in exon 1β); 1 family carrying a predicted benign mutation not previously described that did not segregate among cases (p.P11T located in exon 1α); 2 families with a synonymous change with unknown effect on the 3'UTR of protein p14ARF (c.369C>T located in exon 2), and 1 SMP patient and 1 family carrying intronic mutations.

CDKN2A mutations were detected in 8.5% (20 of 234) of patients with SMP and in 14.1% (46 of 326) of melanoma-prone families. In the set of families, the frequency of *CDKN2A* mutation differs according to the number of patients with melanoma within the family. *CDKN2A* mutation was found in 10.9% (29 of 265), 23.4% (11 of 47), 36.4% (4 of 11), and 66.7% (2 of 3) of families with 2, 3, 4, and 5 cases, respectively (P = .001). Also, statistically significant differences were observed according to the number of FMP in the family. Germline *CDKN2A* mutations were found in 6.3% (14 of 224) of families without FMP cases and in 30.1% (25 of 83) and 40% (6 of 15) of families with 1 or 2 FMP cases, respectively ($P < .001$).

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Table I. Number of primary melanomas in patients with multiple primary melanoma according to cyclin-dependent kinase inhibitor 2A status

	CDKN2A+		*CDKN2A* WT		
Analysis group	**No.**	**%**	**No.**	**%**	***P* value**
All MPM					
2	34	12.6	236	87.4	**<.001**
3	11	21.2	41	78.8	
≥4	12	48.0	13	52.0	
SMP					
2	12	6.3	179	93.7	**.015**
3	5	15.6	27	84.4	
≥4	3	27.3	8	72.7	
FMP					
2	22	27.8	57	72.2	**.033**
3	6	30.0	14	70.0	
≥4	9	64.3	5	35.7	

Statistically significant *P* values are set in bold.
CDKN2A, Cyclin-dependent kinase inhibitor 2A; *FMP*, familial multiple primary melanoma; *MPM*, multiple primary melanoma; *SMP*, sporadic multiple primary melanoma; *WT*, wild type; +, mutation.

Table II. Age at diagnosis of first melanoma according to cyclin-dependent kinase inhibitor 2A status

Analysis group (patients)	**No.**	**Mean age at diagnosis, y**	**SD**	**Adjusted *P***
All				
CDKN2A+	90	39.4	12.8	**<.001**
CDKN2A WT	546	47.2	16.5	
Total	636	46.1	16.3	
Missing data	58			
Familial melanoma				
CDKN2A+	72	40.1	13.6	**.040**
CDKN2A WT	350	45.3	15.9	
Total	422	44.4	15.6	
Missing data	38			
All MPM				
CDKN2A+	53	38.1	11.9	**<.001**
CDKN2A WT	266	50.0	17.1	
Total	319	48.0	16.9	
Missing data	28			
SMP				
CDKN2A+	18	35.8	8.5	**<.001**
CDKN2A WT	196	50.6	17.2	
Total	214	49.4	17.1	
Missing data	20			

Statistically significant *P* values are set in bold.
Bonferroni correction was used.
CDKN2A, Cyclin-dependent kinase inhibitor 2A; *MPM*, multiple primary melanoma; *SMP*, sporadic multiple primary melanoma; *WT*, wild type; +, mutation.

In the subgroup of patients with MPM, we evaluated whether the presence of a *CDKN2A* mutation was related to the number of melanoma developed independent of the familial history of melanoma. We found that the number of tumors correlates with the presence of *CDKN2A* mutations, observing 12.6% of positive mutation carriers in cases with 2 melanomas, and up to 48.0% of mutation carriers in patients who develop at least 4 melanomas. These differences were also detected in both SMP and FMP cases (Table I).

We observed that the presence of *CDKN2A* mutations also modulates the age of onset among the set of patients. Overall, patients with melanoma and *CDKN2A* mutations showed lower age of onset compared with wild type cases (adjusted $P < .001$) (Table II). Such differences were also found when the analyses were focused on patients with familial melanoma (adjusted $P = .040$), all MPM (adjusted $P < .001$), and SMP (adjusted $P < .001$).

Finally, the overrepresentation of other malignances in *CDKN2A*-mutated families was assessed combining the information from the 326 melanoma-prone families and from those families with a SMP case. Overall, the presence of relatives that develop other cancer types was more frequently reported by patients with melanoma carrying *CDKN2A* mutations. We found that the PR of other cancers in germline *CDKN2A* mutated pedigrees was 2.98 (adjusted $P = .012$). The analyses according to cancer type showed an increased presence of pancreatic cancer (PR 2.97, adjusted $P = .006$), lung cancer (PR 3.04, adjusted $P < .001$), and breast cancer (PR 2.19, adjusted $P = .018$) in first- and second-degree relatives of patients with melanoma carrying *CDKN2A* mutations, compared with the wild type. In contrast, no differences were observed in the presence of nephrourologic or colon cancer (Table III). In the analyses restricted to melanoma-prone families (Table III), the association between the presence of pancreatic cancer and lung cancer and *CDKN2A* mutation in the family remained statistically significant (PR 3.26, adjusted $P = .012$ and PR 3.17, adjusted $P < .001$; respectively).

To evaluate whether the increased number of patients with lung cancer within *CDKN2A*-mutant families could be associated with differences related to smoke exposure, we analyzed the smoking habits in 172 individuals from 75 melanoma-prone families. Overall, we did not observe differences in smoking habits, number of cigarettes, age of daily smoking onset, or the number of cigarettes/day between *CDKN2A* mutation carriers vs non-*CDKN2A* mutation carriers or between patients with melanoma and nonaffected individuals. Furthermore, no differences were observed in the smoking habits between the individuals included in our database compared with

Table III. Families with presence/absence of other cancers in first- and second-degree relatives of the patient with melanoma according to the family cyclin-dependent kinase inhibitor 2A status

Analysis including SMP families and melanoma-prone families with at least 2 melanoma cases							
	CDKN2A+ (n = 66)		*CDKN2A* WT (n = 494)				
Other solid tumors in the family	**No.**	**%**	**No.**	**%**	**PR**	**95% CI**	**Adjusted *P***
Other cancers (all)							
Presence	54	88.5	292	69.7	**2.99**	1.40-6.40	**.012**
Absence	7	11.5	127	30.3			
Missing data	5		75				
Pancreatic cancer							
Presence	11	18.0	22	5.3	**2.97**	1.72-5.15	**.006**
Absence	50	82.0	396	94.7			
Missing data	5		76				
Nephrourologic cancer*							
Presence	10	16.4	41	9.8	1.64	0.89-3.03	.738
Absence	51	83.6	376	90.2			
Missing data	5		77				
Lung cancer							
Presence	24	39.3	60	14.4	**3.04**	1.93-4.80	**<.001**
Absence	37	60.7	357	85.6			
Missing data	5		77				
Breast cancer							
Presence	20	32.8	67	16.1	**2.19**	1.36-3.55	**.018**
Absence	41	67.2	349	83.9			
Missing data	5		77				
Colon cancer							
Presence	9	14.3	66	15.8	0.90	0.47-1.74	1.000
Absence	54	85.7	351	84.2			
Missing data	3		77				
Analysis focused only on melanoma-prone families with at least 2 melanoma cases							
	CDKN2A+ (n = 46)		*CDKN2A* WT (n = 280)				
Other solid tumors in the family	**No.**	**%**	**No.**	**%**	**PR**	**95% CI**	**Adjusted *P***
Other cancers (all)							
Presence	37	86.0	177	70.5	2.31	1.01-6.37	.246
Absence	6	14.0	74	29.5			
Missing data	3		29				
Pancreatic cancer							
Presence	9	20.9	13	5.2	**3.26**	1.92-12.14	**.012**
Absence	34	79.1	237	94.8			
Missing data	3		30				
Nephrourologic cancer*							
Presence	9	20.9	26	10.4	1.94	1.02-3.70	.426
Absence	34	79.1	223	89.6			
Missing data	3		31				
Lung cancer							
Presence	18	41.9	36	14.5	**3.17**	1.87-5.39	**<.001**
Absence	25	58.1	213	85.5			
Missing data	3		31				
Breast cancer							
Presence	14	32.6	41	16.5	2.08	1.18-3.67	.114
Absence	29	67.4	208	83.5			
Missing data	3		31				
Colon cancer							
Presence	8	17.8	38	15.4	1.16	0.58-2.32	1.000
Absence	37	82.2	209	84.6			
Missing data	1		33				

Statistically significant *P* values are set in bold.
Bonferroni correction was used.
CDKN2A, Cyclin-dependent kinase inhibitor 2A; *CI*, confidence interval; *PR*, prevalence ratio; *SMP*, sporadic multiple primary melanoma; *WT*, wild type; +, mutation.
*Kidney, bladder, or prostate cancer.

those observed in the general population (data available on request).

DISCUSSION

In this study we have explored the effect of germline mutations in *CDKN2A*, which is the major high-risk melanoma susceptibility gene, in the largest Spanish cohort to our knowledge of patients at high risk (SMP and familial melanoma cases). Overall, we found a slightly increased prevalence of *CDKN2A* mutations in melanoma-prone families than in SMP, consistent with that reported in similar studies from other Mediterranean areas.[34-36]

Previous studies have found an association between the presence of *CDKN2A* mutations within a family and clinical features of the family such as an increased number of cases, or in patients with melanoma, such as an increased number of tumors or a decreased age of onset.[5,37] We also observed an increased number of melanoma cases or number of FMP in Spanish *CDKN2A*-mutated families, an increased number of tumors, and a younger age of onset in patients carrying *CDKN2A* mutations.

The presence of other types of cancer in melanoma-prone families was evaluated regarding the germline status of *CDKN2A*. Overall, the families in which the melanoma cases carried *CDKN2A* mutations showed an increased presence of individuals with other types of cancer. Analyses focused on specific cancer types revealed an association between the presence of germline mutations and cases of pancreatic, lung, and breast cancer. An increased risk of pancreatic cancer was observed in *CDKN2A* mutated families of Caucasian origin.[5,8,10-12,28,34] A multicenter study conducted in a large set of families with 3 or more melanoma cases found a highly increased risk of pancreatic cancer in European families (odds ratio 8.21, 95% confidence interval 2.39-28.24).[5] We detected an increased risk for pancreatic cancer in Spanish melanoma families, even including families with 2 melanoma cases as also reported in French families.[34] Although previous data suggest that germline *CDKN2A* mutations may result in an increased risk of developing other types of cancer,[13] the role of *CDKN2A* in the risk for other cancer types has been less explored. To our knowledge, our study is the largest single-center data set in which the prevalence of other cancers beyond melanoma in *CDKN2A*-mutant families was evaluated. We observed a strong association between the presence of *CDKN2A* mutations and cases of lung cancer within the family. There is a previous study suggesting the association between respiratory cancer and p16-Leiden *CDKN2A* mutation[38] and our group reported a melanoma-prone family in which *CDKN2A* mutations were also present in lung cancer–affected individuals.[13]

In contrast to the association observed with pancreatic or lung cancer, the statistically significant association with breast cancer was restricted to the analyses combining the information from melanoma-prone families and from those families with SMP. The risk of breast cancer in melanoma-prone families carrying *CDKN2A* mutations has been reported previously in North-European populations.[12]

Genetic counseling is increasingly being offered to patients with cancer and/or to their healthy relatives. The genetic testing offered to patients at high risk to develop melanoma may allow us to detect *CDKN2A* mutation carriers who would then be encouraged to practice strategies for melanoma prevention, such as ultraviolet protection, and strategies for early detection. In this study we have found that *CDKN2A*-mutated melanoma families have an increased prevalence of pancreatic, lung, and breast cancer. The effects of cigarette smoking on lung cancer risk is well documented, but also pancreatic cancer[39] and breast cancer[40] have been associated with this risk factor. Furthermore, it has been reported that pancreatic cancer penetrance is higher in smoking *CDKN2A* mutation carriers than in nonsmoker carriers.[41] Our results indicated that the increased prevalence of these cancers observed in *CDKN2A*-mutated families could be explained by genetic factors, when they are exposed to the same environmental factors as the general population.

In conclusion, we have evaluated clinical and family history features related to the presence of germline *CDKN2A* mutations in patients at high risk to develop melanoma and we have observed an increased prevalence of lung, pancreatic, and breast cancer in families carrying *CDKN2A* germline mutations. The data reported in this study may be useful to refine genetic counseling in melanoma and encourage improving cancer prevention programs for *CDKN2A* mutation carriers by adding the recommendation of avoiding smoking in the programs, which already include sun-exposure protection advice and routine total body examination for melanoma early detection. Further studies are needed to identify the best early detection strategies for other cancers in *CDKN2A*-mutant families.

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