ORIGINAL ARTICLE

Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents

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Background: The major factors individually reported to be associated with an increased frequency of CDKN2A mutations are increased number of patients with melanoma in a family, early age at melanoma diagnosis, and family members with multiple primary melanomas (MPM) or pancreatic cancer.
Methods: These four features were examined in 385 families with ⩾3 patients with melanoma pooled by 17 GenoMEL groups, and these attributes were compared across continents.
Results: Overall, 39% of families had CDKN2A mutations ranging from 20% (32/162) in Australia to 45% (29/65) in North America to 57% (89/157) in Europe. All four features in each group, except pancreatic cancer in Australia (p = 0.38), individually showed significant associations with CDKN2A mutations, but the effects varied widely across continents. Multivariate examination also showed different predictors of mutation risk across continents. In Australian families, ⩾2 patients with MPM, median age at melanoma diagnosis ⩽40 years and ⩾6 patients with melanoma in a family jointly predicted the mutation risk. In European families, all four factors concurrently predicted the risk, but with less stringent criteria than in Australia. In North American families, only ⩾1 patient with MPM and age at diagnosis ⩽40 years simultaneously predicted the mutation risk.
Conclusions: The variation in CDKN2A mutations for the four features across continents is consistent with the lower melanoma incidence rates in Europe and higher rates of sporadic melanoma in Australia. The lack of a pancreatic cancer–CDKN2A mutation relationship in Australia probably reflects the divergent spectrum of mutations in families from Australia versus those from North America and Europe. GenoMEL is exploring candidate host, genetic and/or environmental risk factors to better understand the variation observed.

The CDKN2A (MIM# 600160) gene is the major known high-risk cutaneous malignant melanoma (CMM) susceptibility gene. CDKN2A encodes two distinct proteins translated, in alternate reading frames (ARFs), from alternatively spliced transcripts. The α transcript encodes the p16 protein; the smaller β transcript specifies the alternative product p14ARF. Germline CDKN2A mutations have been observed in approximately 20–40% of melanoma-prone families from around the world.[1] Several variables have individually been reported to be associated with an increased frequency of CDKN2A mutations, including increased number of patients with melanoma, early median age at melanoma diagnosis, the occurrence of pancreatic cancer in a family and the occurrence of multiple melanoma tumours in a patient.[2–17] These four features have been assessed separately. However, it has not been previously possible to compare the four factors across geographical regions, nor to examine them simultaneously. Such an evaluation would facilitate interpretation of risks by clinicians from different continents and give a view on putative relationships between genotype and latitude of residence.

The Melanoma Genetics Consortium GenoMEL (http://www.genomel.org), comprising major familial melanoma research groups from North America, Europe, Australia and the Middle East, conducted a study to explore the relationship between these four attributes and the presence of a CDKN2A mutation. All GenoMEL member groups with eligible families participated in this study. The resultant sample of 385 families, 150 of which had CDKN2A mutations, allowed simultaneous evaluation of the four features, as well as inspection of differences by continent.

METHODS

Patients and design

Seventeen GenoMEL centres participated in this study. Families with at least three confirmed patients with melanoma who were screened for mutations in *CDKN2A* (exons 1α, 2 and 3) were eligible for the study. Mutation evaluation, predominantly sequencing or denaturing high performance liquid chromatography followed by sequencing, was conducted at each centre. All families (n = 385) with and without identified mutations

Abbreviations: ARF, alternate reading frame; CMM, cutaneous malignant melanoma; MPM, multiple primary melanoma

were included. CDKN2A mutations that altered the p16 protein were included in the evaluation; a subset of these mutations also influenced p14ARF.

Table 1 presents the number of families and total number of patients with melanoma by study centre. For all centres, written informed consent was obtained from the subjects before participation in the study under Institutional Review Board-approved protocols. The precise methods of ascertainment for the eligible families differed between groups. Details of the participating families from each centre are described elsewhere (see table 1 for references). All melanoma diagnoses were confirmed by review of histological materials, pathology reports, medical records or death certificates. Only patients with confirmed melanomas (invasive or in situ) were included. This restriction may have resulted in differences for some centres in reported numbers of families presented previously. For each family, the absence/presence and type of CDKN2A mutation were reported. Other variables included number of patients with CMM in each family, age at first melanoma diagnosis for each patient, whether or not a patient with CMM had one versus multiple melanoma tumours, and the number of patients with melanoma or first-degree relatives of patients with melanoma in each family who had pancreatic cancer (81% confirmed).

Statistical analysis

Using the data provided, we derived four factors for analysis: number of patients with CMM in a family (#CMM/family), number of patients with CMM in a family with multiple primary melanoma (MPM) tumours, median age at CMM diagnosis in a family (MedAge), and number of patients with pancreatic cancer in a family, combining the reports of pancreatic cancer in patients with melanoma and in first-degree relatives of patients with melanoma. These four factors were evaluated across all 17 GenoMEL groups (total) and within specific geographical regions defined by continent (Australia, Europe and North America). In addition, the four attributes were also individually assessed in the European groups, for which there were sufficient numbers of families (ie, Sweden (Lund+Stockholm), Mediterranean Europe (Barcelona+Genoa+Emilia-Romagna), UK (Leeds+Glasgow), The Netherlands (Leiden) and France (Paris)). The non-parametric Wilcoxon–Mann–Whitney test, Fisher's exact, Jonckheere–Terpstra or Kruskal–Wallis tests, as implemented in the computer programme StatXact (StatXact-4, V.4.0.1), were used to evaluate each of the four factors individually. Multivariate evaluation of the four factors in all families (total) and in families from Australia, Europe or North America was conducted using unconditional logistic regression as implemented in the program Stata (Stata 8.2, V.8.2). It was not possible to evaluate the four factors jointly in groups smaller than that in a continent. For the logistic regression analysis, the presence/absence of a CDKN2A mutation was the dependent variable. We thus measured the association between the ''risk'' of a CDKN2A mutation and the four factors using backward and forward stepwise logistic regression analyses. In addition, the final models were evaluated using likelihood ratio tests. All statistical tests were two sided.

RESULTS

Table 1 presents the number of families, number of patients with melanoma and number of families with CDKN2A mutations by participating centre. There were 385 families with 1720 patients with CMM in this study. Overall, 39% of families (n = 150) had mutations. The frequency of mutations ranged from 20% (32/162) in Australia to 45% (29/65) in North America to 57% (89/157) in Europe. Table 2 shows the CDKN2A mutations by continent and whether the mutation altered p14ARF.

Figures 1–4 show the percentage of families with CDKN2A mutations by number of patients with CMM/family (fig 1), MPM (fig 2), pancreatic cancer (fig 3) and MedAge (fig 4) for each of the four groups (total, North America, Australia and Europe). The frequency of mutations increased significantly as the number of CMM/family increased in each of the four comparison groups (fig 1, p values ranging from $p<0.001$ to $p = 0.004$). In addition, among the mutation-positive families, there were significant differences in the distribution of the number of patients with melanoma per family across continents ($p = 0.002$). In North American and Australian families, the largest increase in mutation frequency was from those families with 5 patients with CMM/family to those with ⩾6 CMM/family

Table 1 Number of families, patients with cutaneous malignant melanoma and families with CDKN2A mutations by study centre and summarised by country/continent and overall (total)

Centre	Country/continent	Total number of families	Total number of patients with CMM	Number of families with CDKN2A mutations	References for family ascertainment
Boston	USA/North America	3	9	0	18
Leeds	USA/North America	1	3	0	29
NCI	USA/North America	38	192	19	19 20
Philadelphia	USA/North America	5	18	1	
Utah	USA/North America	11	150	5	21
Toronto	Canada/North America	7	22	4	
North America		65	394	29	
Barcelona	Spain/Europe	11	38	5	22–25
Emilia-Romagna	Italy/Europe	8	27	1	26
Genoa	Italy/Europe	8	31	5	11
Glasgow	Scotland/Europe	10	31	6	9 27
Leeds	England/Europe	29	107	22	28–30
Leiden	The Netherlands/Europe	26	154	19	31–32
Paris	France/Europe	40	133	19	6 33–34
Lund	Sweden/Europe	15	60	8	12 35
Stockholm	Sweden/Europe	10	36	4	36–38
Europe		157	617	89	
Brisbane	Australia	100	446	18	39–42
Sydney	Australia	62	258	14	10 28 43–44
Australia		162	704	32	
Tel-Aviv	Israel/Asia	1	5	0	
Total		385	1720	150	

CMM, cutaneous malignant melanoma.

Table 2 Number of families with each mutation in CDKN2A by continent

Location of mutation	p16 amino acid or CDKN2A base change	p14ARF amino acid change	Europe (n = 89)	Australia (n = 32)	North America (n = 29)
5′ UTR	c.-34G→T	None	1	1	5
Exon					
1α	c.9_32del24	None		1	
1α	c.18_19ins6	None	1		
1α	c.32_33ins9-32	None	2	2	2
1α	p.W15X	None		1	
1α	c.46delC	None		1	
1α	p.L16P	None	1	2	
1α	p.L16R	None			1
1α	c.52_57dup6	None	1		
1α	p.G23D	None	1		
1α	p.G23R	None	1		
1α	p.R24P	None	5	3	
1α	p.L32P	None	1	3	
1α	p.G35A	None	1	1	
1α	p.A36P	None		1	
1α	p.P38R	None	1		
1α	p.P48L	None	1		
1α	p.P48T	None	1		
1α	p.I49S	None		1	
1α	p.Q50R	None		1	
2	p.V51F	p.G65V		1	
2	p.M53I	p.D68H	6	5	3
2	c.167–197del31	p.1-70p14:70–156p16			1
2	p.R58X	p.P72L			1
2	p.V59G	p.S73R	2		
2	p.L62P	p.A76A	1		
2	p.L63P	p.A77A	1		
2	p.L65P	p.A79A	1		
2	p.G67R	p.R81P	2		
2	p.G67S	p.R81Q	1	1	
2	p.A68L	p.R82L	1		
2	p.E69G	p.G83G	1		
2	p.N71K	p.L86M	2		
2	p.N71S	p.Q85Q		1	1
2	c.225_243del19	p.1–90p14:82–156p16	18	1	1
2	c.240_253del14	p.T95fs	1		1
2	p.R87P	p.P101P			1
2	p.L97R	p.P111P	1		
2	p.R99P	p.P113P	1		
2	p.G101W	p.R115L	10		4
2	c.307_308del2	p.A117fs			1
2	p.D108N	p.R122Q		1	
2	p.R112G	p.P126R	1	1	
2	p.R112_L113insR	p.S127-A128insS	11		
2	p.L113L / p.P114S	p.A128T/ p.A128A	1		
2	p.A118T	p.G132D	1		
2	c.358delG	None	1		
2	p.V126D	None	1		4
2	p.D153spl	p.1–65p14:154–156p16			1
Intron					
2	c.IVS2-105A→G	None	6	3	1
2	c.IVS2+1G→T	p.1–65p14:154–156p16			1

The most frequent mutations for each continent are underlined.

(from 4/9 (44%) to 12/15 (80%) and from 6/24 (25%) to 12/25 patients with (48%), respectively). By contrast, in Europe, the greatest increase in mutation frequency was among families with ⩾4 patients with CMM/family (77%) relative to those with 3 patients with CMM/family (42%). Although all European groups showed patterns consistent with the overall Europe result, only families from Sweden showed a significant association between a mutation and number of patients with melanoma per family ($p = 0.001$).

The frequency of mutations increased significantly as the number of patients with MPM in a family increased for all groups (fig 2, $p<0.001$). We also found significant differences in MPM distribution across continents in mutation-positive families ($p = 0.012$). The presence of one patient with MPM produced a dramatic increase in mutation frequency for North American (0 patients with MPM: 3/30, 10% v ⩾1 patient with MPM: 26/35, 74%) and European families (0 patients with MPM: 18/63, 29% v ⩾1 patient with MPM: 71/94, 76%) The MPM patterns observed in individual European groups were all similar, but significant associations between MPM and mutations were observed only for families from Sweden ($p<0.001$), France ($p = 0.003$) and Mediterranean Europe ($p = 0.006$). By contrast, for Australian families, a striking increase in the mutation frequency required ⩾2 patients with MPM (⩽1 patient with MPM: 13/122, 11% v ⩾2 patients with MPM: 19/40, 48%).

The relationship between pancreatic cancer and CDKN2A mutations showed differences across groups (fig 3). Overall (total), 72% of families with one reported patient with pancreatic cancer had mutations (31/43) and 81% of families with ⩾2 patients with pancreatic cancer had mutations (13/16).

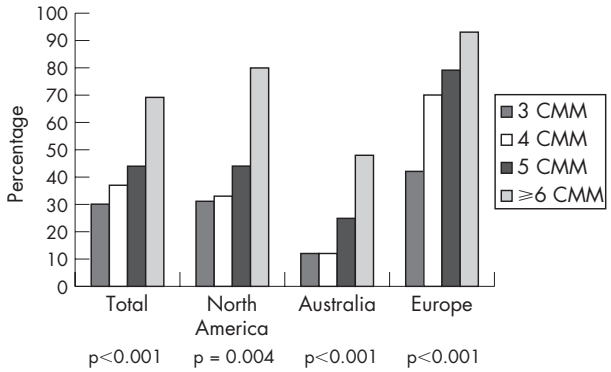

Figure 1 Percentage of families with CDKN2A mutations by number of patients with cutaneous malignant melanoma per family (no. of CMM/family: 3, 4, 5 and ≥6) for each of the four groups (total, North America, Australia and Europe). p Values are shown for each of the four groups.

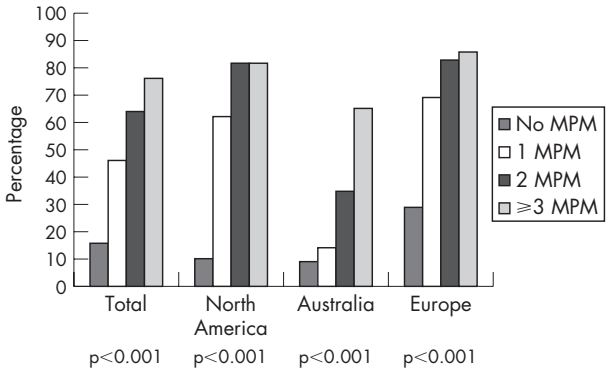

Figure 2 Percentage of families with CDKN2A mutations by number of patients with multiple primary melanoma (MPM: 0, 1, 2 and ≥3) in a family for each of the four groups (total, North America, Australia and Europe). p Values are shown for each of the four groups.

There was a significant association between pancreatic cancer and mutations in families from the total ($p<0.001$), North America ($p = 0.02$) and Europe ($p<0.001$). However, pancreatic cancer was not associated with mutations in Australia ($p = 0.38$). Only three of nine Australian families (33%) with pancreatic cancer had mutations. By contrast, for North American and European families, ≥75% of families with at least one patient with pancreatic cancer had mutations (9/12 and 32/38 families, respectively). In the European families, the strongest evidence for an association between pancreatic cancer and CDKN2A mutations came from The Netherlands ($p = 0.006$), France ($p = 0.007$) and Sweden ($p = 0.04$).

Figure 4 shows the relationship between median age at melanoma diagnosis in a family (MedAge) and CDKN2A mutations using quartiles (<34, 34–40, >40–50 and >50 years) defined among the total. All groups showed a significant decrease in the frequency of mutations as MedAge increased. Again, in the families positive for CDKN2A mutation, there was a significant difference in the distribution of MedAge across continents ($p = 0.002$). The significance resulted from an older median age at melanoma diagnosis in European mutation-positive families compared with either Australian ($p = 0.002$) or North American ($p = 0.03$) families. For European families, a mutation frequency >60% was observed when MedAge was ≤50 years. For Australian families, however, a high mutation frequency (>35%) required an earlier median age at melanoma diagnosis (ie, ≤40 years). North American families showed a pattern intermediate between Europe and Australia, with a step-function decrease in mutation frequency as MedAge increased.

We compared the distributions of #CMM/family, MPM, pancreatic cancer and MedAge across the most frequent CDKN2A mutations, defined as mutations that occurred in ≥5 families (*c.225_243del19, p.M53I, p.G101W, p.R112-L113insR, c.IVS2-105A>G, p.R24P, c.-34G>T, c.32_33ins9-32* and *p.V126D*). At least 70% of families with each of these mutations had ≥1 patient with MPM. For seven of the nine mutations, most of the families had early MedAge. Only *p.R112–L113insR* and *c.-34G>T* had <50% of families with MedAge ≤40 years. The percentage of families with ≥5 patients with CMM/family ranged from 20% to 80%, with five of nine frequent mutations having <40% of families with ≥5 patients with CMM/family. However, there were no significant differences in #CMM/family ($p = 0.14$), MPM ($p = 0.40$) or MedAge ($p = 0.14$) across the nine most frequent mutations. By contrast, the distribution of pancreatic cancer differed significantly across these mutations ($p<0.001$). No families with *p.M53I* (0/14), *c.IVS2-105A>G* (0/10) or *c.32_33ins9–32* (0/6) mutations had pancreatic cancer; 25–36% of families with *p.R24P* (2/8), *c.-34G>T* (2/7) or *p.G101W* (5/14) mutations had pancreatic cancer; finally, pancreatic cancer was observed in ≥60% of families with *p.R112–L113insR* (7/11), *c.225_243del19* (12/20) or *p.V126D* (3/5) mutations. The distribution of pancreatic cancer in families with the most frequent CDKN2A mutations suggested an association with p14ARF (41% of families with a mutation that altered p14ARF had pancreatic cancer *v* 19% of families with a mutation that did not affect p14ARF). However, the observed pattern was not completely consistent. *p.M53I*, a mutation that alters p14ARF had no pancreatic cancer in 14 families; similarly, 60% of families with *p.V126D* had pancreatic cancer, yet this mutation does not alter p14ARF. Additional studies are needed to further evaluate this relationship.

Table 3 shows the multivariate predictors of a CDKN2A mutation for all families (total) and by continent. Given the significant differences observed between continents, we did not constrain the exposed and unexposed/referent categories in the multivariate analysis of the four features to be identical in the continent-specific analyses. There were substantial differences observed across continents. In all groups (total), MedAge ≤50 years, ≥1 patient with MPM and ≥1 patient with pancreatic cancer in a family were significant joint predictors of a CDKN2A mutation. However, #CMM/family was not an independent predictor for mutation risk. For the Australian families, ≥6 patients with melanoma in a family, ≥2 patients with MPM and MedAge ≤40 years simultaneously

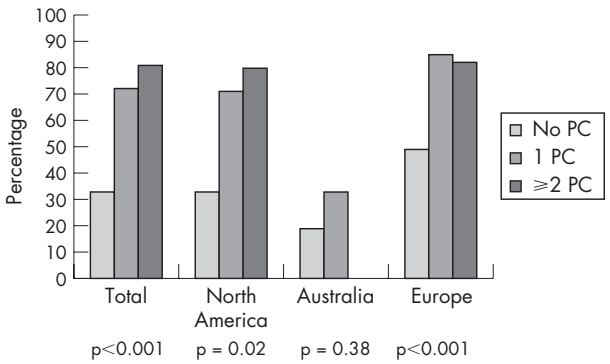

Figure 3 Percentage of families with CDKN2A mutations by number of patients with pancreatic cancer (0, 1 and ≥2) in a family for each of the four groups (total, North America, Australia and Europe). p Values are shown for each of the four groups.

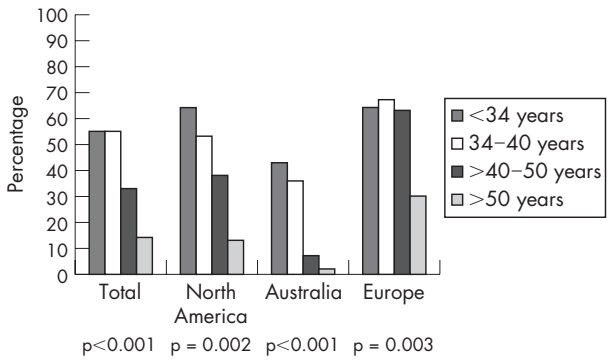

Figure 4 Percentage of families with CDKN2A mutations by median age (in years) at melanoma diagnosis (MedAge: <34, 34–40, >40–50 and >50 years) in a family for each of the four groups (total, North America, Australia and Europe). p Values are shown for each of the four groups.

predicted the risk of a mutation. As was observed in the univariate analyses, pancreatic cancer in a family did not influence the risk for a CDKN2A mutation. For North American families with the smallest sample size of 65 families, only MedAge $\leqslant$ 40 years and $\geqslant$1 patient with MPM were concurrent predictors of a CDKN2A mutation. Finally, for European families, all four factors jointly predicted the mutation risk (table 3). However, the levels for MedAge ($\leqslant$50 years) and #CMM/family ($\geqslant$4) were less stringent than that seen in the other comparison groups.

DISCUSSION

GenoMEL explored the relationship between select risk factors and presence of a CDKN2A mutation in 385 families with at least three confirmed patients with CMM. Individual examination of each of the four attributes showed that for all comparison groups examined (total, North America, Australia and Europe), there were significant associations between CDKN2A mutations and number of patients with melanoma in a family, the occurrence of multiple primary melanoma tumours and median age at melanoma diagnosis in a family. For pancreatic cancer, all groups except Australia showed significant associations with mutations. The frequencies of mutations and the effects of the four attributes, however, varied significantly across continents. Similarly, joint evaluation of the four features showed different predictors of mutation risk across the geographical areas.

Melanoma incidence rates vary widely among Caucasian populations around the world. The incidence rates are highest in Australia (38.5/100 000 for men; 29.5/100 000 for women), intermediate in North America (16.4/100 000 for men; 11.7/100 000 for women) and generally lowest in Western Europe (7.3/100 000 for men; 10/100 000 for women; CANCERMondial: http://www-dep.iarc.fr).[45] Differences in the amount of exposure to ultraviolet radiation, the predominant environmental risk factor for melanoma and variation in host characteristics (eg, hair colour, eye colour, melanocytic nevi, freckling and skin type) may contribute to the wide geographical variation in melanoma incidence rates. In this study, the lowest frequency of CDKN2A mutations was observed in Australia (20%), the area with the highest incidence rates. By contrast, the highest frequency of mutations was observed in Europe (57%), the region with the lowest incidence rates. The underlying difference in incidence rates for melanoma between Australia and Europe is further reflected by the categorisation of the four features in the multivariate analyses. In Australian families (n = 162) in which higher rates of sporadic melanoma occur and fair skin and intense sun exposure predominate, $\geqslant$2 patients with MPM, MedAge $\leqslant$ 40 years and $\geqslant$6 patients with melanoma in a family were needed to concurrently predict CDKN2A mutation risk. For the 157 European families, the factor levels were less stringent (ie, broader), consistent with the lower melanoma incidence rates. Specifically, for the European families, only one patient with MPM, only $\geqslant$4 patients with melanoma in a family and a median age at melanoma diagnosis up to 50 years, in addition to $\geqslant$1 patient with pancreatic cancer, jointly predicted the risk of a CDKN2A mutation. The variation in frequency of CDKN2A mutations by number of patients with melanoma in a family, presence of patients with multiple primary melanoma tumours or pancreatic cancer, and median age at melanoma diagnosis across geographical regions may reflect different distributions of host, genetic or environmental risk factors.

In the univariate analyses, pancreatic cancer was significantly associated with CDKN2A mutations in all regions studied except Australia. As smoking is a well-known risk factor for pancreatic cancer,[46] reported differences in smoking patterns with lower rates in Australia compared with most of Western Europe[47] may contribute to some of the differences seen. However, the rates of smoking in Sweden are lower than that observed in Australia, and pancreatic cancer is strongly associated with CDKN2A mutations in families from Sweden.[12] In addition, there is little difference in pancreatic cancer incidence rates across the regions involved in the current study.

Table 3 Predictors of a CDKN2A mutation from simultaneous evaluation of the four factors (MedAge, multiple primary melanomas, pancreatic cancer, no of cutaneous malignant melanomas/family) across the groups (Total, Australia, North America and Europe)

	Level of variable [OR (95% CI)]			
Variables in final model	**Total**	**Australia**	**North America**	**Europe**
MedAge (years)	$\leqslant$50 [7.42 (3.44 to 16.02)]	$\leqslant$40 [19.62 (5.31 to 72.46)]	$\leqslant$40 [4.04 (1.06 to 15.32)]	$\leqslant$50 [5.15 (1.74 to 15.21)]
MPM	$\geqslant$1 [5.73 (3.40 to 9.68)]	$\geqslant$2 [6.23 (2.20 to 17.60)]	$\geqslant$1 [5.30 (2.42 to 11.65)]	$\geqslant$1 [6.24 (2.72 to 14.32)]
PC	$\geqslant$1 [7.54 (3.42 to 16.67)]	—	—	$\geqslant$1 [8.21 (2.39 to 28.24)]
#CMM/family	—	$\geqslant$6 [6.65 (1.76 to 25.06)]	—	$\geqslant$4 [2.44 (1.05 to 5.64)]
Number of families	385	162	65	157

CMM, cutaneous malignant melanoma; #CMM/family, number of patients with CMM per family; MPM, multiple primary melanomas; PC, pancreatic cancer; dash indicates that the variable did not appear in the final model.
Only the levels of the features and corresponding OR and 95% CI that were significantly associated with the risk of mutation are shown for each group (final model).

The age-standardised incidence rates across the regions range from 6–8/100 000 for men to 5–7/100 000 for women.[45] Alternatively, different distributions of mutations observed in Australia versus other areas may influence the association with pancreatic cancer. Most of the common mutations observed in families from Australia (*p.M53I, c.IVS2–105A>G, p.R24P* or *p.L32P*) have generally shown low frequencies of pancreatic cancer even in non-Australian populations. In the current study, no Australian families with *p.M53I* (n = 5), *c.IVS2–105A>G* (n = 3) or *p.R24P* (n = 3) mutations had pancreatic cancer. Conversely, two of three families with *p.L32P* had pancreatic cancer. Further evaluation of the relationship between these common mutations and pancreatic cancer showed that no non-Australian families with *p.M53I* (0/9) or *c.IVS2–105A>G* (0/7) had pancreatic cancer. Yet, 40% (2/5) of families with *p.R24P* from the UK (2/3) and France (0/2) and 1/1 family from the UK with *p.L32P* had pancreatic cancer. In addition, the CDKN2A mutations (*p.R112–L113insR, c.225_243del19, p.V126D* and *p.G101W*) showing the strongest associations with pancreatic cancer in this and previous studies (summarised in Goldstein[5]) were essentially absent from Australia. Only one Australian family in the current study had any of these four mutations. Thus, the lack of a pancreatic cancer–CDKN2A mutation relationship in Australian families may reflect the spectrum of mutations observed in Australia versus Europe and North America. This finding needs to be confirmed.

The current study had several limitations. The ascertainment and sampling of families at most of the GenoMEL centres was not population-based, and each centre obtained data on extended family members to different degrees. Additionally, the study sample was restricted to families with at least three patients with melanoma, thereby selecting for families at higher risk of melanoma compared with either population-based cases or unselected case series.[17 48] The divergent ascertainment across study sites could, in consequence, have produced variation in the distribution of the four factors of interest. Nonetheless, in the families without CDKN2A mutations, there were no significant differences in the distribution of median age at melanoma diagnosis ($p = 0.54$), patients with multiple primary melanoma tumours ($p = 0.39$) or pancreatic cancer ($p = 0.24$) across the families from North America, Europe or Australia. However, the number of patients with melanoma in a family varied, with European families having significantly fewer patients with melanoma than Australian ($p<0.001$) and North American ($p = 0.009$) families. This difference probably reflects the lower incidence rates of melanoma in Western Europe, and consequently fewer families with large numbers of patients with melanoma. Moreover, it was not possible to fully evaluate the four attributes in areas smaller than a continent. Although continent was selected as the geographical region under study, there is variation in melanoma incidence rates, particularly in the European centres that were part of this study. As such, a continent may not adequately separate families with different risks of melanoma and hence, distributions of risk factors. Finally, as the study was restricted to melanoma-prone families with at least three confirmed patients with CMM, the results from the study may not be applicable to families with only one or two patients with melanoma.

Additional studies are needed to expand the findings from the current study to a wider spectrum of patients with melanoma by evaluating simultaneously the four features examined here in patients with melanoma unselected for a positive family history. A clinic-based study from Boston and Toronto[16] assessed the predictors of a CDKN2A mutation in unselected patients with melanoma, and showed that number of patients with melanoma in a family, median age at melanoma diagnosis and multiple melanoma tumours in a patient were the most important predictors for a mutation. These findings are generally consistent with those observed in the current study. However, given the different joint predictors of mutation risk observed in families from each of the three continents, larger sample sizes and greater geographical diversity are needed to extend these findings to multiple geographical regions. Furthermore, comparison of results from multiple-case families (eg, the current study) and geographically comparable population-based or unselected series of patients could extend the interpretation of risks to different geographical regions and advance the understanding of the relationships between genotype and latitude of residence.

By working together, GenoMEL has produced a dataset to allow simultaneous evaluation of four factors previously individually associated with an increased frequency of CDKN2A mutations. Identification of such features enhances our ability to understand the differential risks that contribute to melanoma. The analyses showed differences in the frequency of CDKN2A mutations according to the number of patients with melanoma in a family, the occurrence of multiple CMM tumours in a patient, early median age at melanoma diagnosis in a family, and the occurrence of pancreatic cancer in a family across geographical regions. The differences may reflect distinct host, genetic or environmental risk factors. GenoMEL is exploring candidate risk factors to better understand the variation observed.

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