Phenotypic and Histopathological Tumor Characteristics According to *CDKN2A* Mutation Status among Affected Members of Melanoma Families

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TO THE EDITOR

Highly penetrant mutations in the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene have been identified as major risk factors for melanoma, and they account for between 20% and 50% of familial cases (Goldstein and Tucker, 2001; Kefford et al., 1999). Pathogenic germline mutations at *CDKN2A* have been associated with malignancies other than melanoma, including breast and pancreatic cancers (Borg et al., 2000; de Snoo et al., 2008; Ghiorzo et al., 2012; Goldstein et al., 2006; Potrony et al., 2014), smoking-related cancers of the head and neck, lung cancer, and gastroesophageal carcinomas (Helgadottir et al., 2014; Potjer et al., 2015), as well as central nervous system tumors (Pasmant et al., 2007; Petronzelli et al., 2001). Moreover, there is recent evidence to suggest that familial melanoma cases who are wild type for *CDKN2A* are not at increased risk for nonmelanoma cancers in contrast to pathogenic mutation carriers (Helgadottir et al., 2014). Distinguishing familial melanoma cases with and without pathogenic *CDKN2A* mutations may serve to heighten awareness of increased risk for other cancers among carriers in melanoma families. Identifying histopathological and other host features that are associated with inherited pathogenic *CDKN2A* mutations may aide in this pursuit and also serve to better characterize melanoma heterogeneity and elucidate important pathobiological differences between carriers and noncarriers of pathogenic *CDKN2A* mutations.

We studied affected members of melanoma families assembled across centers of the GenoMEL consortium and evaluated differences in host and histopathological tumor characteristics between carriers and noncarriers of pathogenic *CDKN2A* mutations. Written informed consent was obtained for each participant, and individual GenoMEL study center investigations were conducted after approval by their respective institutional review boards. To our knowledge, this study is the largest of its kind and incorporates familial melanoma cases from diverse geographical populations.

GenoMEL participants who signed informed consent were asked about their personal melanoma history and to complete a self-administered questionnaire asking about phenotypic characteristics including hair color, eye color, freckling, nevi, burnability (effect of acute sun exposure on skin), and tanning ability (effect of chronic sun exposure on skin). A melanoma family was defined by the presence of three or more cases of verified melanoma, or two cases of verified melanoma in first-degree relatives. Histopathological data were abstracted from pathology or other clinical reports; a centralized pathology review was not performed. Germline DNA was screened for mutations in *CDKN2A* (exons 1α, 1β, 2, and 3) as previously described (Harland et al., 2008), and pathogenicity was assigned according to Supplementary Table S1 online. Pathogenicity was based on demonstrated (i.e., published) impact on the biological functioning of *CDK2NA*, and putative pathogenicity of specific mutations was based on evidence of cosegregation within melanoma families or bioinformatically inferred impact on *CDKN2A* function. Participants were classified based on the presence or absence of a pathogenic or putatively pathogenic variant.

We tested whether differences in levels of histopathological or phenotypic factors exist by *CDKN2A* pathogenic mutation carrier status ($\alpha = 0.05$). Analyses were adjusted for age at diagnosis, gender, study center, and number of affected members per family, and we accounted for the nonindependence of observations arising from familial clustering within the study center using the repeated subject statement. We also adjusted for the presence of any melanocortin-1 receptor variant.

There were 1,928 and 1,696 verified cases with *CDKN2A* genotype data who contributed histopathological and phenotypic data to analyses, respectively. Associations between *CDKN2A* mutational status and age at diagnosis ($P_{trend} < 0.0001$), multiple primary melanomas ($P < 0.0001$), and histologic subtype ($P = 0.003$) were statistically significant after adjustment for covariates and the Bonferroni correction (Table 1). Pathogenic mutation carriers were younger at diagnosis and demonstrated higher proportions of multiple primary melanomas and superficial spreading melanomas compared with wild-type/nonpathogenic mutation carriers. We also observed statistically significant differences between pathogenic and wild-type/nonpathogenic *CDKN2A* mutation carriers with respect to sun burning ($P_{trend} = 0.02$) and skin type ($P = 0.04$) after adjustment for covariates; pathogenic mutation carriers were significantly less likely to develop severe burns with blistering and more likely to report a darker skin type compared with wild-type/nonpathogenic mutation carriers

Abbreviation: CDKN2A, cyclin-dependent kinase inhibitor 2A

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Table 1. Distribution of host and histopathological tumor characteristics among cases of verified cutaneous melanoma belonging to melanoma families[1] overall and according to *CDKN2A* pathogenicity

	Overall with *CDKN2A*	Pathogenic *CDKN2A* mutation carrier	Wild-type or nonpathogenic *CDKN2A* mutation carrier	
	N = 1,928	N = 670	N = 1,258	
	n (%)	n (%)	n (%)	*P*-value[2]
Age at diagnosis				<0.0001
<30 y	367 (19)	169 (25)	198 (16)	
30–39 y	469 (24)	198 (30)	271 (22)	
40–49 y	384 (20)	138 (21)	246 (20)	
50–59 y	362 (19)	95 (14)	267 (21)	
60–69 y	238 (12)	49 (7)	189 (15)	
≥70 y	103 (5)	17 (3)	86 (7)	
Missing	5	4	1	
Multiple primary melanomas				<0.0001
No	1,297 (67)	346 (52)	951 (76)	
Yes	631 (33)	324 (48)	307 (24)	
Missing	0	0	0	
Breslow depth (mm)[3]				0.03
In situ	229 (15)	90 (16)	139 (14)	
0.01–1.00	917 (59)	343 (62)	574 (57)	
1.01–2.00	246 (16)	76 (14)	170 (17)	
2.01–4.00	127 (8)	35 (6)	92 (9)	
>4.00	44 (3)	10 (2)	34 (3)	
Missing	365	116	249	
Histologic subtype				0.003
SSM	879 (71)	378 (73)	501 (70)	
LMM	49 (4)	10 (2)	39 (5)	
NM	104 (8)	31 (6)	73 (10)	
NOS	177 (14)	90 (18)	87 (13)	
Other[4]	24 (2)	6 (1)	18 (3)	
Missing	695	155	540	

Abbreviations: *CDKN2A,* cyclin-dependent kinase inhibitor 2A; LMM, lentigo maligna melanoma; NM, nodular melanoma; NOS, not otherwise specified; SSM, superficial spreading melanomas.

[1]A melanoma family is defined by three or more blood relatives with verified cutaneous melanoma diagnoses or two first degree relatives with verified cutaneous melanoma diagnoses. Verification was made by pathology report (77%), physician letter or clinical document verifying melanoma diagnosis (20%), cancer registry data (3%), or death certificate (<1%). Individuals who were missing data for all histopathological features were excluded from analysis (n = 180).

[2]*P*-value corresponds to a score test with $\alpha = 0.05$ testing for a difference in proportions between wild-type/nonpathogenic and pathogenic *CDKN2A* mutation carriers with respect to a histopathological feature, with adjustment for age at diagnosis (continuous), sex, number of affected members per family, study center, and familial clustering within study center. All analyses were conducted using SAS v.9.3 (SAS Institute, Cary, NC).

[3]Adjusted for the body site of melanoma.

[4]Includes acral lentiginous melanomas and rare subtypes including nevoid, spitzoid, and desmoplastic melanomas.

(Table 2). Neither factor remained significant after the Bonferroni correction. Frequencies and *P*-values for *CDKN2A* association analyses involving all tested histopathological and phenotypic characteristics are reported in Supplementary Tables S2 and S3 online, respectively.

This study reports an analysis of data collected across all GenoMEL centers using a common protocol. Overall, phenotypic and tumor features were similar among affected family members with and without pathogenic mutations in *CDKN2A*. Nevertheless, these groups were differentiated by some of the same factors that distinguish familial melanomas from those arising in the general population (Florell et al., 2005): pathogenic mutation carriers were younger at diagnosis (median age at diagnosis: 38 years vs. 46 years) and they had a greater likelihood of developing multiple melanomas (average number of melanomas: 2.3 vs. 1.4) compared with wild-type/nonpathogenic mutation carriers, findings consistent with results reported by FitzGerald et al. (1996). The preponderance of superficial spreading melanomas observed among pathogenic mutation carriers is consistent with a recent GenoMEL study by Sargen et al. (2015) in which a blinded review of a limited subset of tumors was undertaken.

It has been suggested that heterogeneity within melanoma is due in part to distinct etiologic pathways—one characterized by increased numbers of nevi, lesion presentation on the trunk, and intermittent sun exposure; and one characterized by fewer nevi, lesion presentation on the head and neck, and chronic sun exposure. Our results provide some evidence for differential effects of acute sun exposure between those with and without pathogenic *CDKN2A* mutations and may suggest that pathogenic mutation carriers are less prone to severe sun burns, a result that is consistent with our observation of a higher proportion of darker skin types reported by pathogenic mutation carriers. However, no differences in nevi or body site of lesion were observed.

Notable limitations of our study were as follows: inability to evaluate the impact of inherited variation at other loci on histopathological and phenotypic factors, ascertainment and sampling of families at some centers was not population-based, centers obtained data to varying degrees, and a lack of centralized pathology review. To address the latter limitation, we conducted a sensitivity analysis restricting histopathological data to those reported by dermatopathologists, who are more likely to report on a fuller spectrum of features relevant to melanoma pathology; the results were not appreciably different from those obtained in our main analysis.

In summary, familial cases with and without pathogenic *CDKN2A* mutations exhibit similar distributions of phenotypic and tumor characteristics.