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Lack of germline *PALB2* mutations in melanoma-prone families with *CDKN2A* mutations and pancreatic cancer

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Abstract

The presence of pancreatic cancer (PC) in melanoma-prone families has been consistently associated with an increased frequency of *CDKN2A* mutations, the major high-risk susceptibility gene identified for melanoma. However, the precise relationship between *CDKN2A*, melanoma and PC remains unknown. We evaluated a recently identified PC susceptibility gene *PALB2* using both sequencing and tagging to determine whether *PALB2* might explain part of the relationship between *CDKN2A*, melanoma, and PC. No disease-related mutations were identified from sequencing *PALB2* in multiple pancreatic cancer patients or other mutation carrier relatives of PC patients from the eight melanoma-prone families with *CDKN2A* mutations and PC. In addition, no significant associations were observed between 11 *PALB2* tagging SNPs and melanoma risk in 23 melanoma-prone families with *CDKN2A* mutations or the subset of 11 families with PC or PC-related *CDKN2A* mutations. The results suggested that *PALB2* does not explain the relationship between *CDKN2A*, melanoma, and pancreatic cancer in these melanoma-prone families.

Keywords

CDKN2A; *PALB2*; familial melanoma; pancreatic cancer; germline mutation

Introduction

The *CDKN2A* gene, located on chromosome 9p21, is the major known high-risk melanoma susceptibility gene identified to date. Germline mutations in *CDKN2A* have been observed in 20–40% of melanoma-prone families from around the world (1). The presence of pancreatic cancer (PC) in melanoma-prone families has been consistently associated with an increased frequency of *CDKN2A* mutations (2). However, the precise relationship between *CDKN2A*, melanoma and PC remains unknown. Further, only a subset of *CDKN2A* mutations (e.g. p.R112_L113insR, c.225_243del19, p.G101W, and p.V126D) is linked with the occurrence of PC. Even in melanoma-prone families with putative PC-related *CDKN2A*

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Conflict of interest
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mutations, only a small subset of individuals with these mutations develops PC (3). Thus, factors related to these specific PC-related *CDKN2A* mutations or alternatively non-*CDKN2A* factors may be responsible for the relationship between PC and melanoma in these *CDKN2A* mutation-positive families.

Recently, exomic sequencing identified *PALB2* as a high-risk PC susceptibility gene (4). PALB2 is a binding partner of BRCA2 and plays an important role in facilitating BRCA2's function in repair of DNA double-strand breaks by homologous recombination (5). Previous studies have shown an increased risk of melanoma among *BRCA2* mutation carriers (6). Given the importance of *PALB2* in PC and the relationship between melanoma and PC, we hypothesized that *PALB2* might modify the risk of PC in melanoma-prone families with *CDKN2A* mutations.

Materials and Methods

Study population

The 23 melanoma-prone families included in this study are part of a larger study population that has previously been described (7, 8). Briefly, American families with at least two living first-degree relatives with a history of invasive melanoma were ascertained through health care professionals or self referrals. All diagnoses of melanoma and pancreatic cancer were confirmed by histologic review of pathologic material for melanoma only, or by review of pathology reports, medical records, or death certificates for melanoma and PC. Eleven of these mutation-positive families had at least one member with pancreatic cancer (n=8) or a *CDKN2A* mutation that has been consistently associated with PC (n=3) [p.G101W, p.V126D, c.225-243del19] (9). The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and informed consent was obtained from all participants.

PALB2 sequencing and genotyping

We sequenced 13 *PALB2* exons in available PC patients (n=5) from four melanoma-prone families with *CDKN2A* mutations (Table 1). These 5 PC patients had previously been sequenced for *BRCA2*; no truncating mutations were identified. We also sequenced *PALB2* in seven melanoma patients and/or *CDKN2A* mutation carrier relatives of PC patients from four other melanoma-prone families with *CDKN2A* mutations and PC but in whom DNA from PC patients was not available (Table 1). All forward and reverse sequences were assembled and variants discovered using Variant Reporter™ v1.0 (Applied Biosystems, Foster City, CA). Each variant was then visually confirmed using Sequencher™ v4.0.5 software (Gene Codes Corporation, Ann Arbor, MI).

We tagged *PALB2* for genotyping in the 23 melanoma-prone families with *CDKN2A* mutations [97 melanoma patients; 217 controls (75 spouses and 142 unaffected family members)]. We also separately examined the subset of 11 families with PC or a mutation strongly associated with the occurrence of PC [48 melanoma patients; 116 controls]. Eleven tag SNPs for *PALB2* were selected for genotyping using Fluidigm or Taqman with a minimum minor allele frequency criterion of ≥5% based upon HapMap data for Caucasian (CEU) samples using Tagzilla, software that implements a tagging algorithm based on pairwise linkage disequilibrium (LD) (10). SNPs spanning 20 kb 5′ of the start of transcription (exon 1) up to 10 kb 3′ from the end of the last exon were selected.

Statistical analyses

Conditional logistic regression models adjusted for age, gender, and *CDKN2A* mutation status were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) and the

trend p-value for the association between melanoma and each SNP, using codominant coding for genotypes (0,1,2) with the homozygote of the common allele as the reference group. Conditioning on families was used to account for family ascertainment and differences in disease prevalence among families. While this approach ignores residual familial correlations among family members, it gives estimates that are attenuated toward the null and is thus conservative (11). A gene-based analysis was also performed on *PALB2* to assess the significance of the joint effect of multiple SNPs genotyped. P-values were computed using a rank-truncated test statistic and a permutation-based sampling procedure (20,000 permutations) in the same regression model, taking into account the number of SNPs genotyped and their LD structure (12). All analyses were performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC).

Results and Discussion

We did not observe disease-related mutations from sequencing *PALB2* in all five available PC patients from four *CDKN2A* mutation-positive families. These PC patients had previously also shown no disease-related mutations from sequencing *BRCA2* (unpublished data). In addition, no disease-related mutations in *PALB2* were observed in seven melanoma patients and/or *CDKN2A* mutation carrier relatives of PC patients from four other melanoma-prone families with *CDKN2A* mutations and PC but in whom DNA from PC patients was not available (Table 1). In addition, none of the 11 *PALB2* SNPs were significantly associated with melanoma (Table 2). Further, using the gene-based analysis, *PALB2* was not associated with melanoma (p=0.34). Although based on small numbers, restricting the association analysis to the 11 *CDKN2A* families with PC or PC-related *CDKN2A* mutations again showed no significant associations between melanoma and the 11 *PALB2* SNPs.

Our results are consistent with data from a recent report that found no deleterious *PALB2* mutations in probands from 53 familial melanoma kindreds without *CDKN2A* mutations (13). In addition, recent investigations of *PALB2* mutations in breast-pancreatic cancer families also found similar results showing that mutations of *PALB2* in these families were rare(14, 15). Together, these results suggest that *PALB2* mutations do not account for a substantial proportion of susceptibility in melanoma-prone or breast cancer-prone families with a history of PC.

The major limitation of the current study was the relatively small sample size available for investigation of *PALB2*. Additional examination of *PALB2* in larger samples will be required to conclusively exclude a relationship between *PALB2*, melanoma and *CDKN2A*. Finally, the relationship between *CDKN2A*, melanoma and PC remains unexplained and additional studies are needed to determine the cause(s) for the observed associations.

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Table 1

Subjects sequenced for PALB2 in CDKN2A melanoma-prone families with pancreatic cancer

Family	Affection status of subject(s) sequenced	Relationship of subject(s) sequenced to PC patient in family	No. Melanoma patients in family	No. Pancreatic Cancer patients in family	CDKN2A Mutation		
					Location	Description	Mutation Type
P	CMM/PC	Self	11	1	Exon 2	240-253del14	Frameshift-Chimera
F	CMM/PC	Self	12	1	Exon 2	R87P	Missense
D9	CMM; 357delG carrier	Sibling; offspring	3	1	Exon 2	357delG	Frameshift
J	CMM/PC	Self	7	1	Exon 2	V126D	Missense
K	CMM/PC (n=2)	Self	6	3	Exon 2	V126D	Missense
L	CMM; V126D carrier	Grandchildren (n=2)	10	1	Exon 2	V126D	Missense
AP	CMM	Cousin	3	1	Intron 2	IVS2-105a>g	Splicing
Q	CMM	Offspring (n=2)	3	2	Intron 2	IVS2+1	Splicing

Table 2

Associations of PALB2 tagging SNPs with melanoma in melanoma-prone families with CDKN2A mutations

SNPs	All families (n=23)		Families with PC or PC-related mutations (n=11)	
	OR (95% CI)[1]	p[1]	OR (95% CI) [1]	p[1]
rs240745	0.58 (0.29–1.19)	0.14	0.73 (0.25–2.12)	0.57
rs240744	1.42 (0.59–3.43)	0.44	1.17 (0.39–3.53)	0.78
rs12162020	0.72 (0.33–1.61)	0.43	1.27 (0.44–3.66)	0.66
rs420259	1.30 (0.73–2.32)	0.37	1.60 (0.74–3.48)	0.24
rs513313	1.66 (0.65–4.24)	0.29	1.23 (0.34–4.45)	0.75
rs16940342	1.14 (0.59–2.23)	0.69	1.14 (0.52–2.52)	0.74
rs8058061	0.76 (0.18–3.26)	0.71	1.30 (0.25–6.77)	0.76
rs0843812	0.58 (0.23–1.47)	0.25	0.73 (0.15–3.55)	0.70
rs17806253	0.74 (0.34–1.57)	0.43	0.71 (0.24–2.12)	0.54
rs34514	0.93 (0.49–1.75)	0.82	1.02 (0.40–2.61)	0.96
rs34513	0.88 (0.48–1.61)	0.68	1.24 (0.50–3.04)	0.64

[1]ORs and P values are obtained from likelihood ratio test in conditional logistic regression with melanoma as the outcome variable adjusting for age, gender, and CDKN2A status.