Null Results in Brief

Absence of Deleterious Palladin Mutations in Patients with Familial Pancreatic Cancer

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Abstract

It has been reported that germline mutations in the *palladin* gene (*PALLD*) cause the familial aggregation of pancreatic cancer, but the evidence is weak and controversial. We sequenced the coding regions of *PALLD* in 48 individuals with familial pancreatic cancer. We did not find any deleterious mutations and find no evidence to implicate mutations in *PALLD* as a cause of familial pancreatic cancer. (Cancer Epidemiol Biomarkers Prev 2009; 18(4):1328–30)

Introduction

Brentnall et al. (1) identified a germline missense alteration (P239S) in the palladin gene (*PALLD*) in a familial pancreatic cancer kindred and suggested that this variant may be a cause of the familial clustering of pancreatic cancer. Members of the kindred, known as family X, develop an early onset pancreatic cancer, with pancreatic insufficiency and diabetes mellitus in an autosomal dominant fashion (2) and have significant linkage to chromosome 4q32-34, a region that includes the *PALLD* gene (3). Brentnall et al. (1) implicated an oncogenic function for palladin after finding overexpression of *PALLD* mRNA in pancreatic cancer tissues.

Since this original publication, subsequent studies have not found evidence to link palladin to familial pancreatic cancer (4-8). However, these subsequent studies including linkage analysis, analyses of the *PALLD* P239S variant in familial pancreatic cancer cases, and examination of pancreatic cancer palladin expression have yet to evaluate the full sequence of *PALLD* in patients with familial pancreatic cancer (4-8). To determine if sequence variants in *PALLD* could be contributing to pancreatic cancer susceptibility, we sequenced the entire coding region of *PALLD* in 48 individuals with familial pancreatic cancer.

Materials and Methods

Forty-eight unrelated patients with familial pancreatic cancer, defined as individuals with at least two first-degree relatives with pancreatic cancer, were selected from the National Familial Pancreatic Tumor Registry (9) for analysis. DNA was obtained from EBV-transformed lymphocyte cell lines as previously described (10). PCR of *PALLD* (KIAA0992 Genbank # AB023209.1) was done mainly using primers designed for the pancreatic cancer genome project (11). Sequencing of PCR products was done using the GenomeLab DTCS-Quick Start kit (Beckman-Coulter) according to the kit protocol. Products were sequenced using a CEQ 8000 GeXP Genetic Analysis System (Beckman-Coulter) and the sequence analysis was done with Sequencher v 4.1.4 (Gene Codes Corporation). Putative single nucleotide polymorphisms were evaluated by searching the single nucleotide polymorphism database at the National Center for Biotechnology Information/BLAST Web site. Over 92% of the coding region was successfully sequenced. The study was done with approval from our Institutional Review Board.

Results

No deleterious sequence variants were identified in any of the 48 patients with familial pancreatic cancer (97% confidence interval, 0-7.2%). Five single nucleotide polymorphisms were identified, only one of which changed the amino acid sequence (S236G), the same variant that was identified by Gallinger et al. (5) as a polymorphism having no association with pancreatic cancer. The prevalence of this variant (c.236 A>G) in our study sample was not significantly different (GG, 0.42; AG, 0.44; AA, 0.14) to that previously reported in controls (GG, 0.39; AG, 0.47; AA, 0.14). The remaining variants that we identified were all silent (see Table 1).

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Table 1. PALLD sequence variants

Exon	Nucleotide change	Amino acid change	Frequency	SNP database
2	c. 236 A > G	Ser-Gly	GG = 0.52 AG = 0.35 AA = 0.13	rs62333013
6	c. 467 A > T	Ala-Ala	AA = 0.81 AT = 0.19 TT = 0	No
7	c. 492 A > G	Arg-Arg	AA = 0.90 AG = 0.02 GG = 0.08	rs1059444
12	n. 4019 G > A	3′ UTR	GG = 0.83 GA = 0.16 AA = 0.02	rs1136603
12	n. 4171 C > G	3′ UTR	CC = 0.18 CG = 0.39 GG = 0.46	rs1071738

Abbreviations: SNP, single nucleotide polymorphism; UTR, untranslated region.

Discussion

Pancreatic cancer is a rapidly fatal disease and the fourth leading cause of cancer death in the United States (12). Therefore, considerable efforts under way to discover familial pancreatic cancer genes to help identify individuals at increased risk of developing the disease. Approximately 5% to 10% of pancreatic cancer patients report a family history of pancreatic cancer (13). Germline mutations in several genes including *BRCA2, p16/CDKN2A, LKB1/STK11*, and *PRSS1* have been shown to confer a significant lifetime risk of pancreatic cancer (14-19), but these mutations explain <10% of the familial aggregation of pancreatic cancer. Identifying the genetic basis of familial pancreatic cancer can enable accurate estimation of cancer risk in mutation carriers (20) and enable these carriers to participate in pancreatic screening protocols (21, 22). Early evidence indicates endoscopic ultrasound-based screening is an effective way to identify pancreatic cancer precursor neoplasms (23). Additional research is needed to identify markers that can facilitate the early detection of microscopic neoplasia among individuals undergoing screening (24, 25). Furthermore, identifying the genetic basis of cancer can have therapeutic implications. For example, cancers with inactivation of the BRCA2/Fanconi pathway are sensitive to DNA-crosslinking drugs including mitomycin-C and PARP inhibitors (26, 27).

Brentnall et al. (1) recently reported that germline *PALLD* gene mutations may cause familial pancreatic cancer. A number of follow-up studies on *PALLD* have failed to implicate it as a significant cause of familial pancreatic cancer (4-8). First, linkage analysis of familial pancreatic cancer kindreds from the PacGene consortium (8, 13) and European registries (7) did not identify linkage to the *PALLD* locus, although these studies could not rule out linkage to this region in a minority of families. Second, immunohistochemical labeling has shown that the major splice variant of the palladin protein is not expressed at significant levels in neoplastic cells but is instead expressed in the nonneoplastic stromal cells of pancreatic cancer (6). Third, the sequencing of the entire pancreatic cancer genome failed to identify any somatic mutations in *PALLD* (11) and therefore do not support the role of *PALLD* as a common oncogenic target of pancreatic cancers. Fourth, two searches, one from Europe and one from Canada (4, 5), for the P239S *PALLD* variant in pancreatic cancer families were negative, and additional sequence analyses confined to the exon containing the P239S variant identified only a common missense polymorphism adjacent to P239S (5).

Here, we extend these observation to additional patients and we sequence the entire coding sequence of the *PALLD*, and again, we find no deleterious mutations (5). Our results are consistent with the growing body of evidence that germline alterations in the *PALLD* gene are not a significant contributor to familial pancreatic cancer susceptibility (4-8). It is possible that either germline *PALLD* mutations are a rare cause of familial pancreatic cancer, or that another gene within the 4q32-34 locus contributes to pancreatic cancer susceptibility in family X. Because the family X variant (P239S) and the common polymorphism (S236G) are in close proximity, it is possible that the P239S variant is not functionally deleterious.

In summary, DNA sequence analysis of the *PALLD* gene in familial pancreatic cancers did not identify any deleterious mutations that would support a role for *PALLD* as a familial pancreatic cancer susceptibility gene.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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