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Analysis of the Gene Coding for the *BRCA2*-Interacting Protein *PALB2* in Familial and Sporadic Pancreatic Cancer

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Conflicts of interest
The authors disclose no conflicts.

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Dear Sir:

Current evidence indicates that about 5%–10% of pancreatic cancer has a familial component, although the vast majority of pancreatic cancer families remain unexplained. [1] *PALB2* is a recently identified breast cancer susceptibility gene whose protein is closely associated with BRCA2, and is essential for BRCA2 anchorage to nuclear structures. This functional relationship made *PALB2* a candidate gene for susceptibility to *BRCA2*-related cancers such as pancreas cancer. Recently, Jones et al[2] screened 96 familial pancreatic cancer patients, 16 of whom had 1 first-degree relative with pancreatic cancer and 80 had ≥2 additional relatives with pancreatic cancer, ≥1 of which was first degree.[2] Truncating *PALB2* mutations were identified in 3 patients (3.1%) and there was no difference in average age of cancer onset between mutation-positive and -negative families. We sought to screen a larger cohort of pancreatic cancer cases, including both familial and sporadic types, to determine the wider contribution of *PALB2* gene mutations in pancreatic cancer. We selected a total of 254 individuals with pancreas cancer (148 male, 106 female) at a median age of diagnosis of 61 years. Patients were identified between 1997 and 2007 via clinic-based recruitment in Toronto and Montreal and through either the Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital in Toronto or the population-based Ontario Pancreas Cancer Study. In total, 203 patients were recruited in Toronto and 51 in Montreal. All probands were confirmed to have pancreatic adenocarcinoma by pathology report; 101 pro-bands had a family history of pancreatic cancer, of which 32 had 2 affected first-degree relatives. In these 101 cases, 74 had 1 affected relative, 18 had 2 affected relatives, 7 had 3 affected relatives, and 2 cases had >3 affected relatives (Table 1). Sixty probands had a family history of breast/ovarian cancer, including 21 cases with a family history of pancreatic cancer (included above). Because the cases were ascertained through pancreas cancer studies, the family history of breast cancer or ovarian cancer was not strong, with most families having a single relative affected, and no family having a BRCAPRO score >0.12 (ie, these were not primarily familial breast/ovarian cancer families with 1 or 2 additional cases of pancreas cancer). The median age at diagnosis of pancreatic cancer cases with no family history was 49 years (range, 31–85); 55% of these were <50 years old and 66% were <60 years old at diagnosis. Genomic DNA was obtained from blood, saliva, or buccal cells using standard extraction methods. Before analysis, 20 ng of total genomic DNA was used for whole genome amplification according to the manufacturer's instructions using the Repli-g Mini Kit (Qiagen, Mississauga, Ontario, Canada). We screened the 13 coding exons of *PALB2* by sequencing (n = 83) or high-resolution melt analysis (n = 171), which has similar sensitivity to sequencing.[3] Samples with variants were reamplified by polymerase chain reaction (PCR) using the original, non–whole-genome-amplified DNA as template and the PCR product was sequenced in forward and reverse directions for confirmation. We performed multiplex ligation-dependent probe amplification assay (MLPA) for *PALB2* on 228 samples where we had sufficient DNA of adequate quality, as previously described by Foulkes et al.[4]

We identified a heterozygous, 6.7-kb germline deletion including exons 12 and 13 of *PALB2* in a patient who was affected by breast and then pancreas cancer (ages 47 and 61, respectively) and whose mother died of pancreas cancer at age 83 (Figure 1). This result was confirmed by long range PCR (Takara Bio Inc., Madison, WI) using 2 different primer pairs, which determined the deleted region to span a region from the middle of intron 11 (2.7 kb

from the beginning of exon 12), up to 1.8kb after exon 13. This deletion would disrupt the *PALB2* WD40 motif, which is required for interaction with the BRCA2 protein. [5] Aside from the exonic deletion, 2 previously unreported missense variants (S285L and T911I) were identified, but neither were predicted to be pathogenic. Both these variants were seen in young-onset pancreas cancer cases (41 years and 48 years) with no family history. A number of previously reported variants were also identified (Table 2).

The main purpose of the study by Jones et al[2] was to illustrate that exomic sequencing could be used to identify genes conferring a substantial risk of cancer, in the absence of linkage or other localizing information. After identification of a *PALB2* germ-line mutation in a pancreas cancer case with a family history of pancreatic cancer, truncating mutations were found in 3 other individuals, all of whom had a personal and family history of pancreas cancer. Here, we studied a more varied set of pancreas cancer cases, with fewer probands with very strong family histories of pancreas cancer, but more cases with family histories of other *BRCA2*-related cancers, and 13 cases diagnosed under the age of 40. We found only 1 deleterious mutation, a 2-exon deletion, which is only the second described case of a *PALB2* exonic deletion, the other being reported in a case of Fanconi anemia.[6] To date, *PALB2* exonic deletions have not been reported in breast cancer families.[7]

Taking the 2 sets of data together, we conclude that germ-line mutations in *PALB2* are a rare cause of pancreas cancer, whether hereditary, familial, or sporadic. Of note, 4 of the 5 *PALB2*-related pancreas families identified to date include ≥1 case of breast cancer and in 2 families mutations were seen in individuals with both breast and pancreatic cancer (a total of 9 cases in our study had both breast and pancreas cancer). It may be the case that investigating *PALB2* will be worthwhile in individuals with very strong family histories of pancreas cancer, or where there is a concomitant history of breast cancer, but based on the findings presented herein, routine screening of *PALB2* in individuals with, or at risk of pancreas cancer is difficult to justify. If *PALB2* mutation screening is undertaken, it should include MLPA for exonic deletions. As is seen in breast cancer,[8] the penetrance for these very rare disease-causing variants seems to be high.

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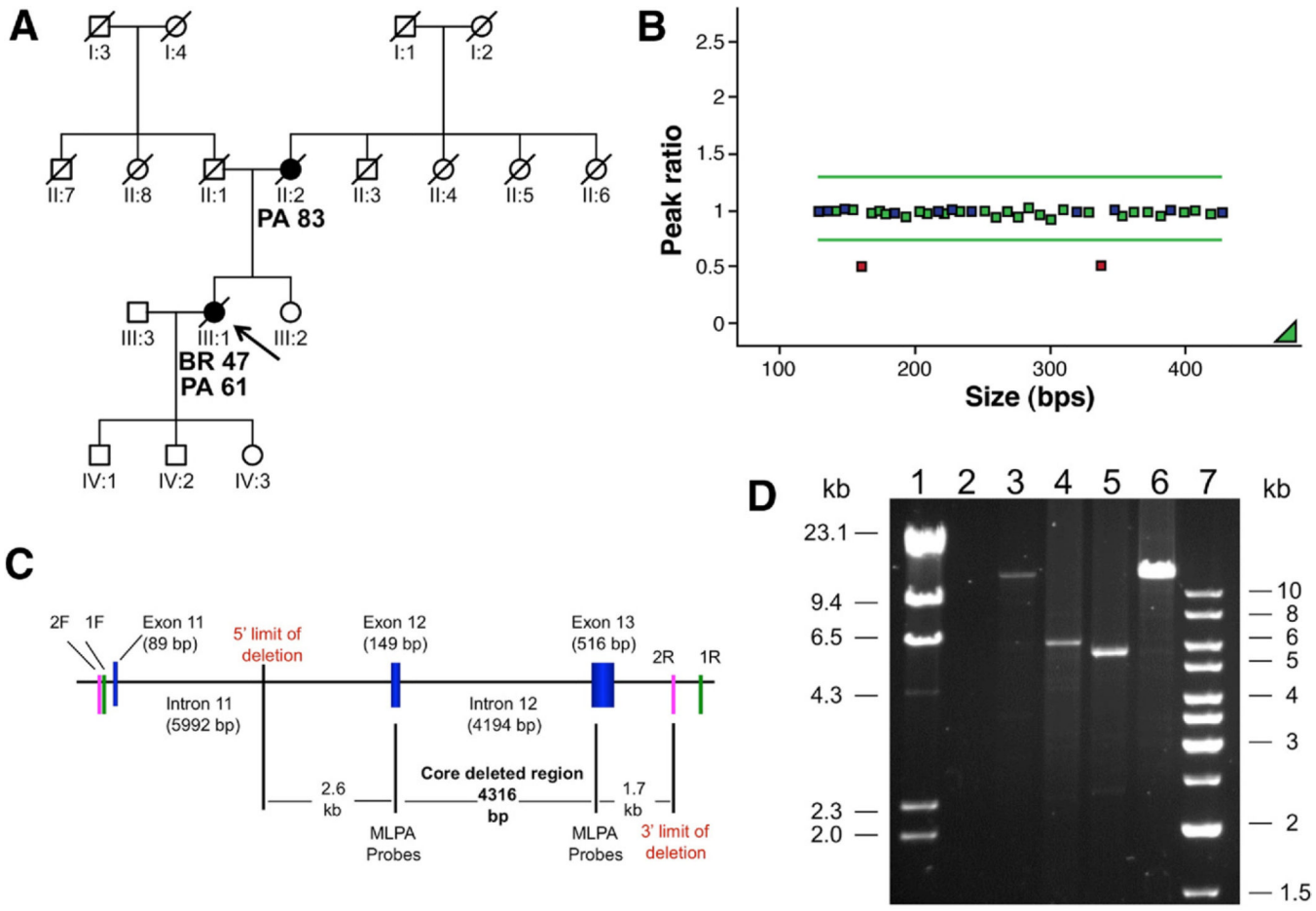

Figure 1.

(*A*) Pedigree of the proband (shown by *arrow*). PA, pancreatic cancer; BR, breast cancer. (*B*) MLPA showing deletion of exon 12 (161-bp fragment) and exon 3 (338-bp fragment). (*C*) Diagram of the deleted region showing the obligate core deletion as defined by the MLPA probes and the potential 5= and 3= limits of the deletion estimated from the observed size of the deleted region by long-range PCR (approximately 6.7–7.0 kb). 1F and 1R, forward and reverse primers for long-range fragment 1; 2F and 2R, forward and reverse primers for long-range fragment 2. (*D*) Long-range PCR, Lane 1, lambda molecular marker; lane 2, no DNA control; lane 3, wt control fragment 1 (expected size 13,141 bp); lane 4, patient fragment 1; lane 5, patient fragment 2; lane 6, wt control fragment 2 (expected size12,633 bp); lane 7, 1 kb molecular marker. Owing to low availability of native genomic DNA, long-range PCR for the patient was performed from whole-genome amplified (WGA) DNA. As a consequence, we expect amplification of large fragments by long-range PCR to be limited by the relative abundance of full-length copies of the region as generated by the WGA reaction. This may explain the absence of the wild-type allele (13 kb) in the patient, who was shown to be heterozygous for the exonic deletion by MLPA, and the correspondingly increased amplification efficiency of the smaller allele carrying the deletion (6 kb).

Table 1

Pancreas Cancer Cases

Type of case	No. of cases (total = 254)	No. of relatives with pancreas cancer	No. of relatives with breast/ovarian cancer
Sporadic pancreas	114	NA	NA
Family history of pancreas cancer	80	1 relative: 74 2 relatives: 18 3 relatives: 7 >3 relatives: 2	NA
Familial pancreas with breast/ovary cases	21		1 relative: 34 2 relatives: 17 3 relatives: 7 >3 relatives: 2
Sporadic pancreas with breast/ovary cases	39	NA	

NA, not applicable.

Table 2

Other Variants Detected in *PALB2*

Sequence variant	Protein change	No. of times observed	Additional information
c.1 – 47 G>A	5′ UTR	13	rs8053188[a]
c.212 –58 A>C	Intron 3	16	non-coding[a]
c.1684 +42 ins.TGA	Intron 4	1	non-coding[c]
c.854 C>T	S285L	1	possibly damaging,[c,d] tolerated[e]
c.1010 T>C	L337S	4	rs45494092[a]
c.1572 A>G	S524S	1	rs45472400[a]
c.1194 G>A	V398V	1	Silent[a]
c.1676 A>G	Q559R	40	rs152451[a]
c.1743 A>G	L581L	1	Silent[c]
c.2014 G>C	E672Q	18	rs45532440[a]
c.2586 +58 C>T	Intron 6	57	rs249954[b]
c.2590 C>T	P864S	1	rs45568339[a]
c.2732 C>T	T911I	1	possibly damaging,[c,d] tolerated[e]
c.2794 G>A	V932M	4	rs45624036[a]
c.2816 T>G	L939W	2	rs45478192[a]
c.2993 G>A	G998E	11	rs45551636[a]
c.3300 T>G	T1100T	16	rs45516100[a]

[a] Previously reported.[9]

[b] Previously reported.[10]

[c] Previously unreported.

[d] Polyphen classification.

[e] Sorting intolerant from tolerant (sift) classification.