Exomic Sequencing Identifies *PALB2* as a Pancreatic Cancer Susceptibility Gene

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There is considerable debate about the value of personal genome sequencing (*1*). In addition to the five individuals whose genomes have been sequenced in their entirety, 68 patients have been evaluated for tumor-specific mutations in all exons of protein-coding genes (exomic sequencing). This coincidentally yielded information about germline sequence variations in these individuals (*2*–*4*). To explore the utility of such information, we evaluated a pancreatic cancer patient (Pa10) whose tumor DNA had been sequenced in (*4*). This patient had familial pancreatic cancer, as defined by the fact that his sister also had developed the disease.

Among the 20,661 coding genes analyzed (*5*), we identified 15,461 germline variants in Pa10 not found in the reference human genome. Of these, 7318 were synonymous, 7721 were missense, 64 were nonsense, 108 were at splice sites, and 250 were small deletions or insertions (54% in-frame). Past studies have shown that tumors arising in patients with a hereditary predisposition harbor no normal alleles of the responsible gene: One allele is inherited in mutant form, often producing a stop codon, and the other (wild-type) allele is inactivated by somatic mutation during tumorigenesis. In Pa10, only three genes met these criteria: *SERPINB12*, *RAGE*, and *PALB2*. Of these, we considered *PALB2* to be the best candidate because germline stop codons in *SERPINB12* and *RAGE*, but not in *PALB2*, are relatively common in healthy individuals and because germline *PALB2* mutations have previously been associated with breast cancer predisposition and Fanconi anemia (*6*), although the function of the gene is not well understood. Pa10 harbored a germline deletion of 4 base pairs (TTGT at ~172 to 175) that produced a frameshift at codon 58; the pancreatic cancer that developed in Pa10 had also somatically acquired a transition mutation (C to T) at a canonical splice site for exon 10 (IVS10+2).

To determine whether *PALB2* mutations occur in other patients with familial pancreatic cancer, we sequenced this gene in a cohort of 96 familial pancreatic cancer patients, 90 of whom were of Caucasian ancestry. Sixteen of these patients had one first-degree relative with pancreatic cancer, and 80 had at least two additional relatives, at least one of whom was first degree, with the disease. Truncating mutations were identified in three of the 96 patients, each producing a different stop codon (Fig. 1). The average age of onset of pancreatic cancer in these families was 66.7 years, similar to the mean age of onset of 65.3 years in the families without *PALB2* mutations. We determined the germline sequence of an affected brother in one of these kindreds, and he harbored the same stop codon. Truncating mutations in *PALB2* are rare in individuals without cancer; none were reported among 1084 normal participants in a previous study that used a cohort of similar ethnicity to ours (*7*). Although some families we identified with a *PALB2* stop mutation had a history of both breast and pancreatic cancer, breast cancer was not observed in all families (pedigrees in fig. S1). From these data, *PALB2* appears to be the second most commonly mutated gene for hereditary pancreatic cancer. The most commonly mutated gene is *BRCA2* (*8*), whose protein product is a binding partner for the *PALB2* protein (*9*).

In summary, through complete, unbiased sequencing of protein-coding genes we have identified a gene responsible for a hereditary disease. This approach is independent of classical methods for gene discovery, such as linkage analysis, which can be challenging in the absence of large families with monogenic diseases. We predict that variations of the approach described here will soon become a standard tool for the discovery of disease-related genes.

References and Notes

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Supporting Online Material

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Materials and Methods
Fig. S1
References

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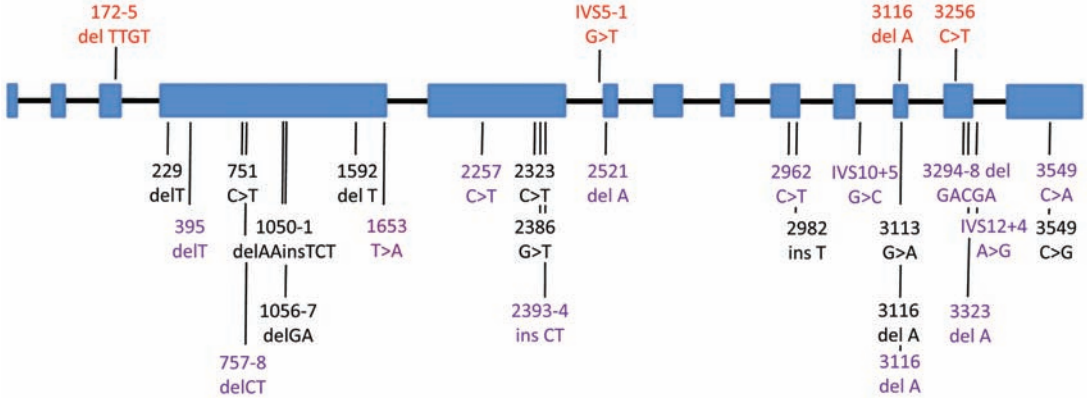

Fig. 1. Location of mutations in the *PALB2* gene. Exons are represented as blue boxes and introns as black lines (not to scale). Mutations previously identified in patients with familial breast cancer or Fanconi anemia are shown in black or purple, respectively. Germline mutations identified in patients with familial pancreatic cancer are shown above the gene in red.

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Supplementary Online Material

Materials and Methods

Study Participants: Patient information, including family history, DNA for genetic analysis, and paraffin embedded tissue for histological examination was collected as part of the National Familial Pancreas Tumor Registry (NFPTR). Informed consent was obtained from all study participants. Approval for this research was obtained from the Institutional Review Board at the Johns Hopkins School of Medicine.

The recruitment and follow-up methods of the NFPTR have been described in detail elsewhere (*S1*). In brief, after informed consent is obtained, a questionnaire is completed by either the pancreatic cancer patient or by a proxy. The questionnaire includes demographic details, cancer history and a three-generation family history. If necessary, families are contacted to clarify questionnaire responses. Whenever possible, all cancers, including the patient's pancreatic cancer and any other cancers reported in the patient or family members, are confirmed from pathology blocks, slides, pathology reports, medical records or death certificates. DNA for sequence analysis was extracted from lymphoblastoid cell lines that were generated from blood samples provided by the patients. DNA from this source was considered to represent the germline. Samples from pancreatic adenocarcinoma patients reporting at least one additional case of pancreatic cancer in their blood relatives were selected for full-sequence analysis. Only one family member from each kindred was tested. Pancreatic cancer patients from families with a documented *BRCA2* gene mutation were excluded.

DNA purification and sequencing: DNA was isolated using DNAeasy kits from Qiagen. The sequencing primers and protocol were identical to those described in (*S2*). Coding variants used to exclude germ-line variations in Pa10 from further consideration were collected from HapMap (http://www.hapmap.org/) or from data obtained through the analysis of the germline of patients with glioblastoma multiforme (*S3*).

Supplemental Results:

In addition to the index case, 96 patients with familial pancreatic cancer underwent full sequence analysis for the *PALB2* gene. The overall prevalence of truncating *PALB2* variants was therefore 3/96 compared to 0/1,084 in Rahman *et al*. (S4) (p=0.0006, Fisher's Exact Test). Controls in the Rahman *et al*.study were from the 1958 British Birth Cohort collection, 97% of whom reported White race. The index case in the current study (Pa10) was Caucasian and not Ashkenazi. Of the 96 other familial pancreatic cancer patients analyzed in this work, 90 (94%) were of Caucasian ancestry; no additional information on country of origin was available for these patients. The NFPTR recruits familial pancreatic cancer patients from across the US. Of the 96 patients, 15 reported Ashkenazi Jewish ancestry (none of which were found to have a truncation in *PALB2*), 59 reported no Ashkenazi Jewish ancestry and the remaining 22 were unknown. In addition to the Rahman *et al.* study, several other studies, in diverse populations (Chinese, Spanish and French-Canadian), have found no *PALB2* stop codons in individuals without cancer or Fanconi anemia (S4-9). The only report of *PALB2* stop mutations in individuals without cancer is a study that showed 0.002% of Finnish individuals carry the c1592delT founder mutation that predisposes to breast cancer in this population. The ages of these founder mutation carriers were ranged from 27 to 51 years, younger than most of the breast cancer patients in the study(S9).

Supplementary Online References

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Figure S1: Pedigrees of pancreatic cancer patients with truncating variants in PALB2. Solid symbols denote individuals with pancreatic cancer and half-shaded symbols denote patients with other types of cancer . The cancer type, age at diagnosis and variant detected is included when known.