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CLINICAL—PANCREAS

Prevalence of Germline Mutations in Cancer Predisposition Genes in Patients With Pancreatic Cancer

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This article has an accompanying continuing medical education activity on page e15. Learning Objective: Upon completion of this exam, successful learners will be able to explain the risks and benefits of genetic screening in patients with pancreatic cancer.

See Covering the Cover synopsis on page 460; see editorial on page 496.

BACKGROUND & AIMS: We investigated the prevalence of germline mutations in *APC*, *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *PRSS1*, *STK11*, and *TP53* in patients with pancreatic cancer. **METHODS:** The Ontario Pancreas Cancer Study enrolls consenting participants with pancreatic cancer from a province-wide electronic pathology database; 708 probands were enrolled from April 2003 through August 2012. To improve the precision of *BRCA2* prevalence estimates, 290 probands were selected from 3 strata, based on family history of breast and/or ovarian cancer, pancreatic cancer, or neither. Germline DNA was analyzed by next-generation sequencing using a custom multiple-gene panel. Mutation prevalence estimates were calculated from the sample for the entire cohort. **RESULTS:** Eleven pathogenic mutations were identified: 3 in *ATM*, 1 in *BRCA1*, 2 in *BRCA2*, 1 in *MLH1*, 2 in *MSH2*, 1 in *MSH6*, and 1 in *TP53*. The prevalence of mutations in all 13 genes was 3.8% (95% confidence interval, 2.1%−5.6%). Carrier status was associated significantly with breast cancer in the proband or first-degree relative ($P < .01$), and with colorectal cancer in the proband or first-degree relative ($P < .01$), but not family history of pancreatic cancer, age at diagnosis, or stage at diagnosis. Of patients with a personal or family history of breast and colorectal cancer, 10.7% (95% confidence interval, 4.4%−17.0%) and 11.1% (95% confidence interval, 3.0%−19.1%) carried pathogenic mutations, respectively. **CONCLUSIONS:** A small but clinically important proportion of pancreatic cancer is associated with mutations in known predisposition genes. The heterogeneity of mutations identified in this study shows the value of using a multiple-gene panel in pancreatic cancer.

Keywords: Cancer Risk; Familial Pancreatic Cancer; Pancreatic Cancer Genetics.

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Pancreatic cancer (PC) is a deadly malignancy. Because symptoms generally signal advanced disease,[1] only approximately 20% present with localized disease that may be amenable to curative surgical resection.[2] As a result, PC carries a bleak prognosis: the 5-year survival rate is 6%, which is only a slight improvement from 2% in the 1970s.[3] Approximately 40,000 Americans will die of PC in 2014, making this malignancy the fourth most common cause of cancer death in men and women.[3] New screening and treatment strategies to reduce deaths from PC are needed urgently.

A subset of PC is caused by highly penetrant germline mutations that cause well-characterized cancer syndromes. Hereditary breast and ovarian cancer syndrome is caused by heterozygous germline mutations in *BRCA1* and *BRCA2*. In addition to breast and ovarian cancer, *BRCA1* and *BRCA2* mutation carriers also face an increased risk of prostate cancer and PC.[4,5] The risk of PC among carriers of *BRCA1* and *BRCA2* mutations has been estimated to be between 4- and 7-fold greater than the general population.[4–6]

***Authors share co-first authorship.**

Abbreviations used in this paper: **GATK, Genome Analysis Toolkit; MMR, mismatch repair; NGS, next-generation sequencing; OPCS, Ontario Pancreas Cancer Study; PC, pancreatic cancer; VUS, variants of unknown significance.**

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Lynch syndrome, also called *hereditary nonpolyposis colorectal cancer*, is caused by heterozygous germline mutations in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. MMR mutation carriers face an increased risk of colorectal, duodenal, endometrial, urogenital tract, brain, hepatobiliary, and pancreatic cancers.[7–9] MMR gene mutation carriers have been estimated to develop PC at approximately 8 times the rate of the general population.[10]

Other familial cancer syndromes and genes impart an increased risk of PC. These include familial adenomatous polyposis, familial atypical multiple mole melanoma syndrome, hereditary pancreatitis, Peutz–Jeghers syndrome, and Li–Fraumeni syndrome, which are caused by mutations in *APC*, *CDK2NA*, *PRSS1*, *STK11*, and *TP53*, respectively.[1,11] Recently, *ATM* and *PALB2*, 2 genes that increase the risk of breast cancer, were found to be associated with familial PC.[12,13]

Identifying germline mutations in genes that increase the risk of PC has important ramifications for the patient and their blood relatives. PC patients carrying these mutations may benefit from experimental and targeted therapies, as proposed with PARP inhibitors and platinum-based chemotherapy in PC patients with *BRCA1* and *BRCA2* mutations.[14,15] Unaffected relatives who also carry mutations may be candidates for investigational PC screening protocols.[16,17] Most importantly, patients and relatives who carry mutations can benefit from well-established targeted extrapancreatic cancer screening protocols and interventions, such as prophylactic mastectomy and salpingo-oopherectomy in *BRCA1* and *BRCA2* carriers,[18,19] and more intensive colorectal cancer screening and management in Lynch and familial adenomatous polyposis syndromes.[20]

The goal of this study was to determine the prevalence of germline mutations in genes that increase the risk of PC in a population-based cohort of PC patients and to determine which clinical characteristics are associated with mutation carrier status.

Materials and Methods

Patients

Probands were selected from the Ontario Pancreas Cancer Study (OPCS), which has been described previously.[21] The OPCS is a population-based registry that contacts all patients in Ontario with a pathologic diagnosis of pancreatic ductal adenocarcinoma from a province-wide electronic pathology reporting system. Consenting participants answer questionnaires, agree to a review of medical records, and provide biospecimens (blood, saliva, and access to biopsy specimens and resections). OPCS probands recruited between April 2003 and August 2012 with available blood or saliva samples were included in this study. The research ethics board of Mount Sinai Hospital approved this study.

Patient Sampling Procedure

We aimed to detect at least 1 mutation for any gene with mutations in at least 1% of pancreatic cancer. To achieve this goal with at least 95% power, a sample size of 290 patients was selected. The subset of 290 probands was selected randomly from the OPCS using a stratified random sampling strategy to maximize the precision for the population estimate of *BRCA2* mutation prevalence because *BRCA2* was the only gene that we expected to be mutated frequently. Family history of pancreatic, breast, or ovarian cancer was expected to modulate the prevalence of *BRCA2* mutations, so probands were stratified according to family history of cancer. The 3 strata were as follows: PC in a first-, second-, or third-degree relative; breast or ovarian cancer in a first-, second-, or third-degree relative without a family history of PC; or no family history of pancreatic, breast, or ovarian cancer in a first-, second-, or third-degree relative. The sampling weight for each strata was defined such that it minimized the variance estimate of the *BRCA1* and *BRCA2* mutation prevalence estimates following the approach proposed by Choi and Briollais.[22] Probands with known mutations were included in the randomization. A control subject without a history of cancer was sequenced along with the 290 PC probands to assist with variant filtering.

Next-Generation Sequencing and Bioinformatics

Genomic DNA was extracted from peripheral blood lymphocytes using organic solvent isolation or column-based purification methods. A custom targeted capture kit was designed using NimbleDesign (NimbleGen, Inc, Madison, WI) that targeted the exonic and splice site regions of 385 genes previously associated with cancer for use in the clinical laboratory at Mount Sinai Hospital. Libraries were created using the SeqCap EZ Library (NimbleGen, Inc) and KAPA Library Preparation Kits (Kapa Biosystems, Inc, Wilmington, MA) according to the manufacturers' protocols. Next-generation sequencing NGS was performed on Illumina HisSeq 2500 platforms (Illumina, Inc, San Diego, CA). Bases were called with default settings using Illumina BCLFAST2 Conversion Software (v.1.8.4; Illumina, Inc). Sequencing reads were aligned to the reference genome hg19 using the Burrows–Wheels Aligner (v.0.6.2-r126).[23] Picard (v1.79; available: http://picard.sourceforge.net; accessed, February 1, 2014) removed duplicate reads. The Genome Analysis Toolkit (GATK) (v2.0-25-gf27c683)[24] was used to detect single-nucleotide substitutions and small insertions and deletions, using best practices from the GATK website (available: http://www.broadinstitute.org/gatk/; accessed, February 1, 2014). To maximize sensitivity to detect variants, no variant quality filters were applied. ANNOVAR[25] and in-house scripts annotated variants.

Variant Characterization

Variants in *APC*, *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *PRSS1*, *STK11*, and *TP53* were considered for analysis if they were: (1) called nonreference by GATK; (2) predicted to affect the protein sequence or the splice site (ie, ±5 base pairs); (3) had an allele frequency of less than 1% in the 1000 Genome project,[26] dbSNP138,[27] and the National Heart, Lung, and Blood Institute Exome Variant Server ESP6500 data set (available: http://evs.gs.washington.edu/EVS/; accessed, February 1, 2014); and (4) were not present in the control subject who was sequenced simultaneously with the cohort.