Gastroenterology 2019;156:1905–1913

BASIC AND TRANSLATIONAL—PANCREAS

Prevalence of Germline Mutations Associated With Cancer Risk in Patients With Intraductal Papillary Mucinous Neoplasms

Michael Skaro,[1] Neha Nanda,[1] Christian Gauthier,[1] Matthäus Felsenstein,[1] Zhengdong Jiang,[1] Miaozhen Qiu,[1,3,4] Koji Shindo,[1] Jun Yu,[2,5] Danielle Hutchings,[1] Ammar A. Javed,[5] Ross Beckman,[5] Jin He,[5] Christopher L. Wolfgang,[1,2,5,6] Elizabeth Thompson,[1,2,6] Ralph H. Hruban,[1,2,6] Alison P. Klein,[1,2,6,7] Michael Goggins,[1,2,6,8] Laura D. Wood,[1,2,6] and Nicholas J. Roberts[1,2,6]

[1]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [3]Department of Medical Oncology, Sun Yat-Sen University Cancer Center; State Key Laboratory of Oncology in South China; [4]Collaborative Innovation Center for Cancer Medicine, Guangzhou, China; [5]Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [6]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [7]Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; and [8]Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

BACKGROUND & AIMS: Many patients with pancreatic adenocarcinoma carry germline mutations associated with increased risk of cancer. It is not clear whether patients with intraductal papillary mucinous neoplasms (IPMNs), which are precursors to some pancreatic cancers, also carry these mutations. We assessed the prevalence of germline mutations associated with cancer risk in patients with histologically confirmed IPMN. **METHODS:** We obtained nontumor tissue samples from 315 patients with surgically resected IPMNs from 1997 through 2017, and we sequenced 94 genes with variants associated with cancer risk. Mutations associated with increased risk of cancer were identified and compared with individuals from the Exome Aggregation Consortium. **RESULTS:** We identified 23 patients with a germline mutation associated with cancer risk (7.3%; 95% confidence interval, 4.9–10.8). Nine patients had a germline mutation associated with pancreatic cancer susceptibility (2.9%; 95% confidence interval, 1.4–5.4). More patients with IPMNs carried germline mutations in *ATM* ($P < .0001$), *PTCH1* ($P < .0001$), and *SUFU* ($P < .0001$) compared with controls. Patients with IPMNs and germline mutations associated with pancreatic cancer were more like to have concurrent invasive pancreatic carcinoma compared with patients with IPMNs without these mutations ($P < .0320$). **CONCLUSIONS:** In sequence analyses of 315 patients with surgically resected IPMNs, we found that almost 3% to carry mutations associated with pancreatic cancer risk. More patients with IPMNs and germline mutations associated with pancreatic cancer had concurrent invasive pancreatic carcinoma compared with patients with IPMNs without these mutations. Genetic analysis of patients with IPMNs might identify those at greatest risk for cancer.

Keywords: Pancreas; Cancer; Genetics; Predisposition.

Pancreatic adenocarcinoma (PDAC) is a deadly disease, with a 5-year survival rate of just 8%.[1] By 2030, PDAC is predicted to become the second-leading cause of cancer-related death in the United States.[1] Understanding the genetics and biology of pancreatic tumorigenesis is key to early diagnosis when patient outcomes are much improved.[2,3] In particular, understanding the risk factors driving development of noninvasive pancreatic precursor lesions and their transition to invasive carcinoma is essential to appropriate patient stratification and intervention.

Approximately 10% of patients with PDAC have a germline mutation in an established pancreatic cancer susceptibility gene, including *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *CPA1*, *MLH1*, *MSH2*, *PALB2*, *PMS2*, *PRSS1*, and *STK11*.[4–12] The prevalence of a germline mutation is higher still in patients with PDAC and a family history of pancreatic cancer in a first-degree relative, reaching 15%–20%.[4] Inheritance of a germline mutation in an established pancreatic cancer susceptibility gene can affect patient care in several ways. First, knowledge of germline status allows for informed, risk-appropriate screening strategies to be undertaken and PDAC to be detected early.[3,13] Second, because many established susceptibility genes predispose to tumors in a number of organs, recommended screening for these extrapancreatic cancers can be instituted.[14] Finally, in some patients with PDAC, germline mutation status may have therapeutic implications, for example, use of poly [ADP-ribose] polymerase-1 (PARP-1) inhibitors or platinum-based chemotherapy for tumors deficient in homology-directed DNA due to *BRCA2* loss and use of immunotherapy for patients with tumors deficient in mismatch repair due to loss of *MLH1*, *MSH2*, *MSH6*, or *PMS2*.[15–17]

PDAC forms when normal ductal epithelium acquires sequential genetic, cellular, and morphologic alterations.[18–21] These alterations are well defined and result in progression from normal epithelium, to noninvasive precursor lesion,

Abbreviations used in this paper: bp, base pair; ExAC, Exome Aggregation Consortium; IPMN, intraductal papillary mucinous neoplasm; MAF, minor allele frequency; PDAC, pancreatic adenocarcinoma; PARP-1, poly [ADP-ribose] polymerase-1.

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2019.01.254

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Germline mutations associated with cancer risk are frequently found in patients with pancreatic adenocarcinoma. The prevalence of germline mutations in patients with intraductal papillary mucinous neoplasms (IPMNs), a pancreatic cancer precursor lesion, is unknown.

NEW INFORMATION

Almost 3% of patients with IPMN carry a germline mutation associated with pancreatic cancer risk. More patients with IPMNs and germline mutations associated with pancreatic cancer had concurrent invasive pancreatic carcinoma compared to patients with IPMNs without these mutations.

LIMITATIONS

This study included only patients with surgically resected IPMN. Additional studies are needed to determine applicability to IPMN patients not undergoing resection.

IMPACT

Genetic analysis of patients with IPMNs might identify those at greatest risk for cancer.

and finally to invasive carcinoma.[22] Premalignant, noninvasive precursor lesions are of 3 types: microscopic pancreatic intraepithelial neoplasia, macroscopic intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms.[23] Because IPMNs are macroscopic and noninvasive, they represent an ideal opportunity for intervention before progression to PDAC. IPMNs, however, are common in the population,[24,25] and numerous clinical criteria are used as surrogates of high-grade dysplasia or invasive cancer to identify IPMNs patients with a high risk of progression to PDAC who may benefit from surgical intervention. These include size of the main pancreatic duct, cyst size, presence of a mural nodule, and symptoms such as pancreatitis or jaundice.[26–29] Although useful, these clinical criteria are imprecise and indirect measures of tumor biology. Molecular markers that indicate a need for surgical resection are desperately needed but are currently lacking.

Several lines of evidence suggest a possible underlying genetic predisposition to IPMNs. First, IPMNs are often multifocal, and the remnant pancreas is at increased risk of IPMN after resection. This multifocality could be due to intraluminal spread of neoplastic cells, an environmental exposure, an underlying genetic predisposition.[30–32] Second, germline mutations in pancreatic cancer susceptibility genes such as *BRCA2*, *CDKN2A*, and *STK11* have been identified in patients with IPMNs.[33–35] Third, in 1 screening study of 78 patients at high risk of pancreatic cancer, most of the patients who had pancreatic resection for concerning imaging findings had IPMNs.[36] And in another study, the prevalence of incipient and high-grade IPMN was higher in patients with familial compared with sporadic PDAC.[37] Finally, several reports have suggested that patients with an IPMN have an increased risk of developing other cancers, including colon cancer.[35,38–41]

Despite the potential ramifications of germline status in patients with IPMNs, no studies have systematically characterized germline mutations in this patient population. Therefore, we used targeted next-generation sequencing to characterize variation in the genes that predispose to PDAC and other cancers in a series of 315 patients with surgically resected, histologically confirmed IPMN.

Materials and Methods

Patients and Biospecimens

This study was reviewed and approved by the Johns Hopkins Medicine Institutional Review Board. A total of 350 unselected patients with surgically resected IPMN and available nontumor tissue samples were identified from surgical and pathology databases. Where available, 25 mg of fresh-frozen nontumor tissue (duodenum) was obtained. Otherwise, 0.6-mm tissue cores were obtained from formalin-fixed blocks of nontumor tissue (duodenum, gallbladder, liver, or spleen).

DNA Extraction

DNA was extracted from fresh-frozen nontumor tissue by using the DNeasy Blood & Tissue Kit (catalog no. 69504, Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA from formalin-fixed, paraffin-embedded nontumor tissue cores was extracted by using the QIAamp DNA FFPE Tissue Kit (catalog no. 56404, Qiagen) and deparaffinization solution (catalog no. 19093, Qiagen) with the following protocol modifications: 1) 10 or fewer tissue cores were deparaffinized with 120 μL of deparaffinization solution, and 11 or more tissue cores were deparaffinized with 200 μL of deparaffinization solution; 2) after the addition of ATL buffer and proteinase K, samples were incubated for up to 7 days with intermittent mixing by inversion and vortex, and 3) an additional 20 μL of proteinase K was added to the sample after 48 hours of incubation. Extracted DNA was quantified with the Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA) with the Qubit 1x dsDNA BR Assay Kit (catalog no. Q32853, Thermo Fisher Scientific).

Library Preparation, Sequencing, and Analysis

DNA sequence libraries for each sample were prepared with the TruSight Rapid Capture Kit (catalog no. FC-140-1105, Illumina, San Diego, CA) and pooled into groups of 12 before capture with the TruSight Cancer probe set (catalog no. FC-140-1101, Illumina) according to the manufacturer's instructions. The TruSight Cancer probe set covers the coding region of 94 hereditary cancer predisposition genes (Supplementary Table 1). Fragment size and yield of captured libraries were assessed with the Bioanalyzer 2100 Instrument (catalog no. G2939BA, Agilent, Santa Clara, CA) with the High Sensitivity DNA Kit (catalog no. 5067-4626, Agilent) and the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) with the Qubit 1× dsDNA HS Assay Kit (catalog no. Q33230, Thermo Fisher Scientific). Captured sequence libraries were further pooled into groups of 24 samples and sequenced on the Illumina MiSeq System (Illumina) using the

MiSeq Reagent Kit v2 (300 cycles) (catalog no. MS-102-2002, Illumina), generating 150 base pair (bp) paired-end reads. Sequence reads were processed through a standardized pipeline by using MiSeq Reporter Software v2.6 (Illumina). Sequence reads were aligned to the human reference genome (hg19) with Burrows-Wheeler aligner.[42] Variant calling was performed with the Genome Analysis Tool Kit.[43] Samples with less than 20× average target coverage were excluded from analysis. Annotation of variants was conducted with ANNOVAR and included amino acid alterations based on Reference Sequence Database (RefSeq) transcripts, minor allele frequency (MAF) with publicly available variant databases (1000 Genomes Project, Exome Variant Server, and Exome Aggregation Consortium [ExAC]), and ClinVar annotations.[44–46] Variants (single base substitutions (SBSs) or insertions/deletions [indels]) within exons or adjacent intronic sequence (±1, ±2) of target genes were classified as either benign, of unknown significance, or deleterious germline mutation as follows: 1) benign: a variant of any functional consequence of >0.5% MAF or a synonymous variant of any MAF; 2) variant of unknown significance: a missense SBS or in-frame indel of ≤0.5% MAF; and 3) deleterious: a frameshift or splicing indel, a nonsense SBS, a stop loss SBS, or splicing SBS of ≤0.5% MAF. Sequence reads supporting deleterious germline variant calls were inspected with the Integrative Genomics Viewer.[47]

Variant Validation

Putative deleterious germline mutations were validated via polymerase chain reaction (PCR) amplification and Sanger sequencing of the variant region. Primers (Integrated DNA Technologies, San Jose, CA) used for amplification are given in Supplementary Table 2. PCR setup was conducted with OneTaq (catalog no. M0480S, New England Biolabs, Ipswich, MA) according to manufacturer's instructions. Amplification was conducted with the T100 Thermo Cycler (catalog no. 1861096, Bio-Rad, Hercules, CA) with the following cycling conditions: 1 cycle of 94°C for 30 s, 21 cycles of 94°C for 30 s, 70°C for 30 s (decrement 0.5°C per cycle), 68°C for 60 s, and 25 cycles of 94°C for 30 s, 60°C for 30 s, 68°C for 60 s. PCR products were purified with the QIAquick PCR Purification Kit (catalog no. 28104, Qiagen) and Sanger sequenced (Genewiz, South Plainfield, NJ). Sequence chromatograms were visualized with 4Peaks (Nucleobytes, Amsterdam, The Netherlands)

Statistical Analysis

Statistical analyses were conducted with Prism 6 (GraphPad Software, San Diego, CA). Confidence intervals for percentage of samples with a hereditary cancer predisposition gene or pancreatic cancer susceptibility gene were calculated with the modified Wald method. Germline mutations in surgically resected IPMN patients and non–The Cancer Genome Atlas samples from ExAC were grouped by gene and compared with a 2-tailed chi-square test with Yates correction. Bonferroni correction for multiple testing was used, and a P value $< 5.3 \times 10^{-4}$ was considered significant. Germline mutations in patients with surgically resected IPMN and unselected PDAC patients were grouped by gene and compared by using a 2-tailed Fisher exact test. Clinicopathologic variables in surgically resected IPMN patients by the presence of germline mutation and invasive cancer were compared with a 2-tailed Fisher exact test, except for age at time at surgery, duration of follow-up, and mean longest diameter of IPMN, which were compared using a 2-tailed, unpaired t test. P values $< .05$ were considered significant. P values less than 0.0001 were abbreviated to $<.0001$.

Results

A total of 350 patients with surgically resected IPMNs were included in this study. Of these, 315 patients had greater than 20 times the average target coverage after sequencing and were included in subsequent analyses. There were 138 patients with high-grade IPMN (43.8%) and 152 patients with low- or intermediate-grade IPMN (48.3%), and 25 did not have a reported grade (7.9%). There were 62 (19.7%) patients with multifocal IPMN and 72 patients with IPMN and a co-occurring invasive carcinoma (22.9%), most commonly PDAC (57 patients). Other types of invasive carcinoma present in the study population included colloid carcinoma (11 patients), adenosquamous PDAC (1 patient), anaplastic carcinoma (1 patient), colloid carcinoma and PDAC (1 patient), and signet ring carcinoma (1 patient). Forty patients (12.7%) had a family history of pancreatic cancer in either a first- or second-degree relative, and 54 patients (17.1%) had a personal history of cancer. Further details of patient demographics and characteristics are given in Table 1 and Supplementary Table 3.

Targeted sequencing generated a mean of 150 mega base pairs (Mbp) per sample (range, 10–562 Mbp; standard deviation, 138 Mbp). Mean target coverage was 256 times (range, 20–877 times; standard deviation, 140 times). Mean target regions covered at 1 time and 10 times were 99.1% (73.9%–100%, standard deviation, 2.0%) and 97.2% (range, 46.9%–100%; standard deviation, 5.6%) respectively. The mean number of single nucleotide variants identified per patient was 276 (range, 56–340; standard deviation, 40), and the mean number of insertions and deletions was 1 (range, 1–3; standard deviation, 0).

Variants identified in the 94 hereditary cancer predisposition genes covered by the TruSight Cancer Panel were classified as either benign variant, variant of unknown significance, or deleterious germline mutations (see Materials and Methods). This analysis identified 26 germline mutations in 23 patients (7.3%; 95% confidence interval [CI], 4.9–10.8) (Table 2). Ten germline mutations in 9 patients were in established pancreatic cancer susceptibility genes (2.9%; 95% CI, 1.3%–5.4%), including 5 germline mutations in *ATM*, 3 germline mutations in *BRCA2*, 1 germline mutation in *MSH6*, and 1 germline mutation in *PALB2*. One germline mutation was also identified in *BUB1B*, a previously identified candidate pancreatic cancer susceptibility gene.[11] More than 1 patient had a germline mutation involving *ATM* (5 patients), *BRCA2* (3 patients), *FANCI* (2 patients), and *PTCH1* (2 patients). Three patients had more than 1 germline mutation in a hereditary cancer predisposition gene. One patient had both an *RB1* and *PTCH1* germline mutation, 1 patient had both a *BRCA2* and *FANCM*

Table 1. Demographics and Characteristics of Patients With Surgically Resected IPMN

Characteristic	n	%
Race		
White	270	85.7
Other	45	14.3
Sex		
Male	162	51.4
Female	153	48.6
Age, *y*		
<40	7	2.2
41–45	6	1.9
46–50	11	3.5
51–55	17	5.4
56–60	28	8.9
61–65	40	12.7
66–70	60	19.0
71–75	69	21.9
76–80	49	15.6
81–85	21	6.7
>86	7	2.2
Family history of pancreatic cancer		
Yes	40	12.7
No	205	65.1
NR	70	22.2
Personal history of cancer		
Yes	54	17.1
No	247	78.4
NR	14	4.4
Diagnosis		
IPMN	243	77.1
IPMN and invasive carcinoma	72	22.9
Size of IPMN, *cm*		
<1	22	7.0
≥1 and <2	87	27.6
≥2 and <3	85	27.0
≥3 and <4	48	15.2
≥4 and <5	23	7.3
≥5	32	10.2
NR	18	5.7
Number of IPMNs		
1	253	80.3
2+	62	19.7
Duct type		
Branch duct	146	46.3
Main duct	112	35.6
NR	57	18.1
Grade of IPMN		
High	138	43.8
Low or intermediate	152	48.3
NR	25	7.9

NOTE. Family history of pancreatic cancer in first- and second-degree relatives.
IPMN, intraductal papillary mucinous neoplasm; NR, not reported.

germline mutation, and another had both a *BRCA2* and *MSH6* germline mutation. Similar findings have been reported for familial pancreatic cancer and familial pancreatitis in which affected individuals have deleterious germline mutations in multiple susceptibility genes.[11,48]

We next compared the prevalence of germline mutations in surgically resected IPMN patients to similarly analyzed, publicly available variant data from ExAC (Table 3).[46] Germline mutations were not significantly enriched when we considered all sequenced hereditary cancer predisposition genes ($P = .6590$) or pancreatic cancer susceptibility genes ($P = .1403$). Similarly, the majority of individual genes sequenced were not significantly enriched in patients with an IPMN. However, 3 genes were significantly enriched after Bonferroni correction for multiple testing. These genes are *ATM* ($P < .0001$), *PTCH1* ($P < .0001$), and *SUFU* ($P < .0001$).

We also compared the prevalence of germline mutations in established pancreatic cancer susceptibility genes between surgically resected IPMN patients and previously published series of unselected PDAC patients (Supplementary Table 4).[8,9] No genes analyzed had statistically significant overrepresentation or underrepresentation in surgically resected IPMN patients compared with unselected PDAC patients.

The patients with IPMN who had a germline mutation in a pancreatic cancer susceptibility gene were more likely to have concurrent invasive carcinoma than IPMN patients without a germline mutation. Specifically, 5 of 9 patients with germline mutation in a pancreatic cancer susceptibility gene had concurrent invasive carcinoma compared with 67 of 306 patients without a germline mutation (Fisher exact test, $P = .0320$) (Table 4). There was no statistically significant association between a germline mutation in a hereditary cancer predisposition gene and concurrent invasive carcinoma (Table 4). Of the 5 patients with a germline mutation in a pancreatic cancer susceptibility gene and invasive carcinoma, only 1 had a family history of pancreatic cancer in a first- or second-degree relative, and none had a reported previous cancer history. Otherwise, there were no statistically significant differences between IPMN patients with a germline line mutation in either a hereditary cancer predisposition gene or a pancreatic cancer susceptibility gene compared with IPMN patients without a germline mutation with respect to family history of pancreatic cancer in first- or second-degree relatives, personal history of cancer, age at surgery, sex, presence of multifocal IPMN, high-grade dysplasia, size, or main duct involvement (Table 4).

Patients with IPMN and invasive carcinoma were significantly more likely to have high-grade dysplasia ($P < .0001$) and involvement of the main pancreatic duct ($P < .0059$) compared with patients without concurrent invasive carcinoma (Supplementary Table 5). There were no other statistically significant associations between IPMN patients with and without invasive carcinoma.

Follow-up was available for 243 of 315 patients, with a mean duration of 33.3 months (range, 0.1–199.3 months). The number of patients with a new diagnosis of pancreatic cancer during follow-up was 2 (0.8%). There were no significant differences in mean duration of follow-up or incident pancreatic cancers between patients with a germline mutation and those without a germline mutation (Table 4).

Table 2. Germline mutations identified in patients with surgically resected IPMNs

Patient number	Gene	Type	Transcript	Germline mutation			Functional consequence	Concurrent invasive carcinoma
1	*ATM*	Pancreatic cancer	NM_000051	g.chr11:108098600_G>A	c.G170A	p.W57X	Stopgain	Signet ring carcinoma
2	*ATM*	susceptibility gene	NM_000051	g.chr11:108117812_CAAAG>C	c.1024_1027del	p.K342fs	Frameshift deletion	PDAC
3	*ATM*		NM_000051	g.chr11:108137985_C>T	c.C2554T	p.Q852X	Stopgain	—
4	*ATM*		NM_000051	g.chr11:108175549_C>T	c.C5644T	p.R1882X	Stopgain	PDAC
5	*ATM*		NM_000051	g.chr11:108206686_A>T	c.A8266T	p.K2756X	Stopgain	—
6	*BRCA2*		NM_000059	g.chr13:32907014_A>T	c.A1399T	p.K467X	Stopgain	—
7	*BRCA2*		NM_000059	g.chr13:32914437_GT>G	c.5946delT	p.S1982fs	Frameshift deletion	—
8	*BRCA2*		NM_000059	g.chr13:32972346_TTGTA>T	c.9697_9700del	p.C3233fs	Frameshift deletion	Colloid carcinoma
6	*MSH6*		NA	g.chr2:48033791_GTAAC>G	—	—	Splicing	—
9	*PALB2*		NM_024675	g.chr16:23649206_GACAA>G	c.172_175del	p.L58fs	Frameshift deletion	PDAC
10	*ALK*	Hereditary cancer	NM_004304	g.chr2:29436851_G>A	c.C3742T	p.R1248X	Stopgain	—
11	*BRIP1*	predisposition gene	NM_032043	g.chr17:59871059_C>A	c.G1372T	p.E458X	Stopgain	Adenosquamous PDAC
12	*BUB1B*		NM_001211	g.chr15:40462282_C>T	c.C199T	p.R67X	Stopgain	PDAC
13	*CDH1*		NM_001317184	g.chr16:68771344_C>A	c.C26A	p.S9X	Stopgain	—
14	*FANCA*		NA	g.chr16:89871687_C>G	—	—	Splicing	—
15	*FANCD2*		NM_001018115	g.chr3:10083368_C>T	c.C757T	p.R253X	Stopgain	PDAC
16	*FANCI*		NM_001113378	g.chr15:89838165_C>T	c.C2476T	p.Q826X	Stopgain	—
17	*FANCI*		NM_018193	g.chr15:89843584_C>CA	c.2678dupA	p.Q893fs	Frameshift insertion	—
8	*FANCM*		NM_001308133	g.chr14:45645855_G>T	c.G3820T	p.E1274X	Stopgain	Colloid carcinoma
18	*NBN*		NM_002485	g.chr8:90960063_T>A	c.A1903T	p.K635X	Stopgain	—
19	*PTCH1*		NM_001083603	g.chr9:98279098_TC>T	c.4delG	p.E2fs	Frameshift deletion	Colloid carcinoma
20	*PTCH1*		NM_001083603	g.chr9:98279098_TC>T	c.4delG	p.E2fs	Frameshift deletion	—
20	*RB1*		NA	g.chr13:48922000_G>A	—	—	Splicing	—
21	*RECQL4*		NM_004260	g.chr8:145739410_G>A	c.C1960T	p.Q654X	Stopgain	—
22	*SUFU*		NM_001178133	g.chr10:104268965_CA>C	c.223delA	p.R75fs	Frameshift deletion	—
23	*WT1*		NM_000378	g.chr11:32456755_GC>G	c.136delG	p.A46fs	Frameshift deletion	—

NOTE. Genomic co-ordinates use hg19 version of human genome.
c, transcript change; chr, chromosome; g, genomic change; IPMN, intraductal papillary mucinous neoplasm; p, protein change associated with germline mutation; PDAC, pancreatic adenocarcinoma.

Table 3. Comparison of germline mutations identified in patients with surgically resected IPMN and ExAC controls

	IPMN			EXAC			
Germline mutation	AC	AN	AF	AC	AN	AF	*P*
Hereditary cancer predisposition gene	26	630	0.041	3921	105,586	0.037	.6590
Pancreatic cancer susceptibility gene	10	630	0.016	992	105,732	0.009	.1403
ATM	5	630	0.008	134	106,203	0.001	<.0001*
BRCA2	3	630	0.005	216	106,188	0.002	.2858
MSH6	1	630	0.002	261	106,196	0.002	.9709
PALB2	1	630	0.002	63	106,206	0.001	.8413
ALK	1	630	0.002	24	106,209	0.000	.3570
BRIP1	1	630	0.002	120	106,202	0.001	.7336
BUB1B	1	630	0.002	32	106,209	0.000	.4874
CDH1	1	630	0.002	9	96,677	0.000	.0861
FANCA	1	630	0.002	117	105,585	0.001	.7189
FANCD2	1	630	0.002	83	106,209	0.001	.9947
FANCI	2	630	0.003	83	106,208	0.001	.1569
FANCM	1	630	0.002	174	106,183	0.002	.9746
NBN	1	630	0.002	59	103,676	0.001	.7286
PTCH1	2	630	0.003	14	105,834	0.000	<.0001*
RB1	1	630	0.002	6	106,198	0.000	.0235
RECQL4	1	630	0.002	173	105,674	0.002	.9754
SUFU	1	630	0.002	0	105,586	0.000	<.0001*
WT1	1	630	0.002	13	105,241	0.000	.1476

AC, germline mutation allele count; AF, frequency of germline mutations; AN, assessed allele number; ExAC, Exome Aggregation Consortium; IPMN, intraductal papillary mucinous neoplasm.
*Significant when applying Bonferroni correction for multiple testing (threshold for significance = 5.3×10^{-4}).

Table 4. Comparison of patients with surgically resected IPMN with and without a germline mutation

	Germline mutation in hereditary cancer predisposition gene			Germline mutation in pancreatic cancer susceptibility gene		
	+	−		+	−	
Variable	(n = 23)	(n = 292)	*P*	(n = 9)	(n = 306)	*P*
Patients with concurrent invasive carcinoma (n)	9	63	.0694	5	67	.0320
Patients with family history of pancreatic cancer (n)	6	34	.0971	3	37	.1670
Patients with personal history of cancer (n)	1	53	.1419	1	53	1.0000
Mean age at surgery (*y*)	65.2	68.2	.1911	62.2	68.2	.1025
Male patients (n)	14	148	.3916	6	156	.5031
Patients with high-grade dysplasia (n)	8	130	.6442	2	136	.6865
Mean longest diameter of IPMN (*cm*)	2.1	2.7	.0986	2.1	2.7	.3674
Patients with multifocal IPMN (n)	4	58	1.0000	2	60	.6921
Patients with main duct involvement (n)	6	106	1.0000	2	110	.3078
Mean duration of follow-up (*mo*)	46.8	32.5	.1248	40.2	33.2	.6287
Incident pancreatic cancer during follow-up (n)	0	2	1.0000	0	2	1.0000

NOTE. Not all patients had a grade of dysplasia assigned. *P* values calculated by using samples with reported family history status (6/19, 34/226, 3/9, 37/236), reported personal cancer history (1/20, 53/281, 1/9, 53/292), grade assigned (8/19, 130/271, 2/6, and 136/284), main duct involvement (6/15, 105/243, 2/6, and 110/252), and incident pancreatic cancer during follow-up (0/15, 2/229, 0/6, and 2/238).

Discussion

In this retrospective study of patients with surgically resected, histologically confirmed IPMN, we found that 7.3% of patients had a germline mutation in a hereditary cancer predisposition gene and that 2.9% had a germline mutation in an established pancreatic cancer susceptibility gene. The numbers of patients with a germline mutation in a either a hereditary cancer predisposition gene or a pancreatic cancer susceptibility gene were not significant compared with ExAC controls. However, the prevalence of a germline mutation in pancreatic cancer susceptibility genes in IPMN patients is similar to that in recent studies of PDAC patients unselected for family history, in which between 3.9% and 5.5% of patients had a germline mutation.[8,9]

Three individual genes were significantly enriched in surgically resected IPMN patients compared with ExAC controls. These genes include *ATM* (5 germline mutations), *PTCH1* (2 germline mutations), and *SUFU* (1 germline mutation). *ATM* is a serine/threonine kinase integral to DNA double-strand break repair in response to ionizing radiation.[49] *ATM* is an established pancreatic cancer susceptibility gene, and recent evidence suggests that *ATM* germline mutations are among the most common found in familial and sporadic PDAC patients.[8,9,11,50] *PTCH1* and *SUFU* are both components of the Hedgehog signaling pathway. *PTCH1* is a transmembrane protein that suppresses Hedgehog signaling when not bound to ligand, and *SUFU* is a cytoplasmic protein that inhibits Hedgehog signaling through binding of GLI transcription factors.[51] Germline mutations in *PTCH1* and *SUFU* are implicated in Gorlin syndrome and predisposition to childhood medulloblastoma.[52–54] *PTCH1* and *SUFU* are intriguing candidate pancreatic cancer susceptibility genes, because aberrant Hedgehog signaling has been implicated in pancreatic tumor development. Specifically, overexpression of SHH is observed in more than 70% of pancreatic tumors and results in autocrine-mediated changes to the tumor microenvironment.[55,56] Furthermore, *PTCH1* and *SUFU* can be somatically mutated in PDAC.[11,57–59] Additional large-cohort studies of IPMN and PDAC patients will be needed to determine the prevalence of *PTCH1* and *SUFU* germline mutations and risk of tumor development.

Patients with surgically resected IPMN with a germline mutation in a pancreatic cancer susceptibility gene were significantly more likely to have concurrent invasive pancreatic carcinoma than patients without a germline mutation (Table 4). The majority of patients with a germline mutation in a pancreatic cancer susceptibility gene and invasive carcinoma did not have a reported family history of pancreatic cancer (4 of 5 patients) or personal cancer history (5 of 5 patients). This may indicate that the presence of a germline mutation in a pancreatic cancer susceptibility gene is an independent risk factor for progression to PDAC. Prospective studies, however, are necessary to determine the magnitude of any increased risk.[60]

Recent studies have suggested that knowledge of germline status in PDAC patients may be of limited personal utility, except for guiding use of PARP-1 inhibitors and immunotherapies in patients with defects in homology-directed and mismatch DNA repair, respectively.[15–17] Knowledge of germline status in patients with an IPMN, however, may be advantageous. Specifically, IPMN patients with a germline mutation may warrant additional surveillance to diagnose pancreatic and extrapancreatic tumors, as is the case for germline mutation carriers with a family history of PDAC.[61,62] Additional prospective studies are needed to confirm that additional screening in this patient population improves early diagnosis rates and patient outcomes.

Our study has several limitations. First, this is a retrospective study of patients with surgically resected IPMN. Although this ensured that all IPMNs were histologically confirmed, these patients are a subset of all patients with IPMN. Specifically, our study included patients with IPMNs advanced enough to warrant surgery and, therefore, may be more likely to develop or have already developed PDAC. Assessment of unselected patients is necessary to determine the clinical utility of stratification by germline mutation status in patients with IPMN who have not yet had surgical resection. Second, although we present the largest characterization of hereditary cancer predisposition genes in IPMN patients to date, our sample size is too small to detect associations with germline mutations that are a rare cause of IPMN or PDAC. Third, we used publicly available data from ExAC for controls, because a large data set of similarly sequenced controls was not available. Variant data from ExAC samples was similarly annotated and analyzed against IPMN cases; however, sequencing methodology was different, and this may result in batch effects that hinder the analysis of gene associations. Fourth, only limited clinicopathological data were available; therefore, associations between cancer risk factors (other than those presented in the study) and germline mutation status could not be explored.

In conclusion, we characterized germline mutations in hereditary cancer predisposition genes in surgically resected IPMN patients. We found that germline mutations were most frequently identified in *ATM* and *BRCA2* and that germline line mutations in *ATM*, *PTCH1*, and *SUFU* were significantly more common in patients with an IPMN than in ExAC controls. Furthermore, IPMN patients with a germline mutation in a pancreatic cancer susceptibility gene were significantly more likely to have concurrent invasive pancreatic carcinoma. Our study indicates that germline testing of IPMN patients is warranted and may have important implications for patient care.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.01.254.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7–30.

2. Amundadottir LT. Pancreatic cancer genetics. Int J Biol Sci 2016;12:314–325.
3. **Canto MI**, **Almario JA**, Schulick RD, et al. Risk of neoplastic progression in individuals at high risk for pancreatic cancer undergoing long-term surveillance. Gastroenterology 2018;155:740–751.
4. Chen F, Roberts NJ, Klein AP. Inherited pancreatic cancer. Chin Clin Oncol 2017;6:58.
5. Hu C, Hart SN, Bamlet WR, et al. Prevalence of pathogenic mutations in cancer predisposition genes among pancreatic cancer patients. Cancer Epidemiol Biomarkers Prev 2016;25:207–211.
6. **Grant RC**, **Selander I**, Connor AA, et al. Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer. Gastroenterology 2015;148:556–564.
7. Holter S, Borgida A, Dodd A, et al. Germline BRCA mutations in a large clinic-based cohort of patients with pancreatic adenocarcinoma. J Clin Oncol 2015;33:3124–3129.
8. Shindo K, Yu J, Suenaga M, et al. Deleterious germline mutations in patients with apparently sporadic pancreatic adenocarcinoma. J Clin Oncol 2017;35:3382–3390.
9. Hu C, Hart SN, Polley EC, et al. Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. JAMA 2018;319:2401–2409.
10. **Hu C**, **LaDuca H**, Shimelis H, et al. Multigene hereditary cancer panels reveal high-risk pancreatic cancer susceptibility genes. JCO Precision Oncol 2018:1–28.
11. Roberts NJ, Norris AL, Petersen GM, et al. Whole genome sequencing defines the genetic heterogeneity of familial pancreatic cancer. Cancer Discov 2016;6:166–175.
12. Tamura K, Yu J, Hata T, et al. Mutations in the pancreatic secretory enzymes CPA1 and CPB1 are associated with pancreatic cancer. Proc Natl Acad Sci U S A 2018;115:4767–4772.
13. Tanaka M. Intraductal papillary mucinous neoplasm of the pancreas as the main focus for early detection of pancreatic adenocarcinoma. Pancreas 2018;47:544–550.
14. Hruban RH, Canto MI, Goggins M, et al. Update on familial pancreatic cancer. Adv Surg 2010;44:293–311.
15. Villarroel MC, Rajeshkumar NV, Garrido-Laguna I, et al. Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer. Mol Cancer Ther 2011;10:3–8.
16. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–413.
17. O'Reilly EM, Lee JW, Lowery MA, et al. Phase 1 trial evaluating cisplatin, gemcitabine, and veliparib in 2 patient cohorts: Germline BRCA mutation carriers and wild-type BRCA pancreatic ductal adenocarcinoma. Cancer 2018;124:1374–1382.
18. Hruban RH, Goggins M, Parsons J, et al. Progression model for pancreatic cancer. Clin Cancer Res 2000;6:2969–2972.
19. Notta F, Chan-Seng-Yue M, Lemire M, et al. A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns. Nature 2016;538:378–382.
20. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013;339:1546–1558.
21. Makohon-Moore AP, Matsukuma K, Zhang M, et al. Precancerous neoplastic cells can move through the pancreatic ductal system. Nature 2018;561:201–205.
22. Pittman ME, Rao R, Hruban RH. Classification, morphology, molecular pathogenesis, and outcome of premalignant lesions of the pancreas. Arch Pathol Lab Med 2017;141:1606–1614.
23. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. Nat Rev Dis Primers 2016;2:16022.
24. Laffan TA, Horton KM, Klein AP, et al. Prevalence of unsuspected pancreatic cysts on MDCT. AJR Am J Roentgenol 2008;191:802–807.
25. Lennon AM, Wolfgang CL, Canto MI, et al. The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? Cancer Res 2014;74:3381–3389.
26. Tanaka M, Chari S, Adsay V, et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 2006;6:17–32.
27. Vege SS, Ziring B, Jain R, et al. American gastroenterological association institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. Gastroenterology 2015;148:819–822.
28. Tanaka M, Fernandez-del Castillo C, Adsay V, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. Pancreatology 2012;12:183–197.
29. Tanaka M, Fernandez-Del Castillo C, Kamisawa T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. Pancreatology 2017;17:738–753.
30. Matthaei H, Norris AL, Tsiatis AC, et al. Clinicopathological characteristics and molecular analyses of multifocal intraductal papillary mucinous neoplasms of the pancreas. Ann Surg 2012;255:326–333.
31. Kang MJ, Jang JY, Lee KB, et al. Long-term prospective cohort study of patients undergoing pancreatectomy for intraductal papillary mucinous neoplasm of the pancreas: implications for postoperative surveillance. Ann Surg 2014;260:356–363.
32. Pea A, Yu J, Rezaee N, et al. Targeted DNA sequencing reveals patterns of local progression in the pancreatic remnant following resection of intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg 2017;266:133–141.
33. Martinez de Juan F, Reolid Escribano M, Martinez Lapiedra C, et al. Pancreatic adenosquamous carcinoma and intraductal papillary mucinous neoplasm in a CDKN2A germline mutation carrier. World J Gastrointest Oncol 2017;9:390–396.
34. Sato N, Rosty C, Jansen M, et al. STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol 2001;159:2017–2022.
35. Lubezky N, Ben-Haim M, Lahat G, et al. Intraductal papillary mucinous neoplasm of the pancreas:

associated cancers, family history, genetic predisposition? Surgery 2012;151:70–75.
36. Canto MI, Goggins M, Hruban RH, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol 2006;4:766–781.
37. Shi C, Klein AP, Goggins M, et al. Increased prevalence of precursor lesions in familial pancreatic cancer patients. Clin Cancer Res 2009;15:7737–7743.
38. Panic N, Capurso G, Attili F, et al. Risk for colorectal adenomas among patients with pancreatic intraductal papillary mucinous neoplasms: a prospective case-control study. J Gastrointestin Liver Dis 2015;24:445–450.
39. Khan S, Sclabas G, Reid-Lombardo KM. Population-based epidemiology, risk factors and screening of intraductal papillary mucinous neoplasm patients. World J Gastrointest Surg 2010;2:314–318.
40. Reid-Lombardo KM, Mathis KL, Wood CM, et al. Frequency of extrapancreatic neoplasms in intraductal papillary mucinous neoplasm of the pancreas: implications for management. Ann Surg 2010;251:64–69.
41. Benarroch-Gampel J, Riall TS. Extrapancreatic malignancies and intraductal papillary mucinous neoplasms of the pancreas. World J Gastrointest Surg 2010;2:363–367.
42. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009; 25:1754–1760.
43. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–1303.
44. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
45. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature 2015; 526:68–74.
46. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285–291.
47. Robinson JT, Thorvaldsdottir H, Winckler W, et al. Integrative genomics viewer. Nat Biotechnol 2011;29:24–26.
48. LaRusch J, Whitcomb DC. Genetics of pancreatitis. Curr Opin Gastroenterol 2011;27:467–474.
49. Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. Nat Rev Mol Cell Biol 2008;9:759–769.
50. Roberts NJ, Jiao Y, Yu J, et al. ATM mutations in patients with hereditary pancreatic cancer. Cancer Discov 2012; 2:41–46.
51. Wu F, Zhang Y, Sun B, et al. Hedgehog signaling: from basic biology to cancer therapy. Cell Chem Biol 2017; 24:252–280.
52. Wolter M, Reifenberger J, Sommer C, et al. Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 1997;57:2581–2585.
53. Pastorino L, Ghiorzo P, Nasti S, et al. Identification of a SUFU germline mutation in a family with Gorlin syndrome. Am J Med Genet A 2009;149A: 1539–1543.
54. **Waszak SM**, **Northcott PA**, Buchhalter I, et al. Spectrum and prevalence of genetic predisposition in medulloblastoma: a retrospective genetic study and prospective validation in a clinical trial cohort. Lancet Oncol 2018; 19:785–798.
55. **Thayer SP**, **di Magliano MP**, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425:851–856.
56. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 2014;25:735–747.
57. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399–405.
58. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015;518:495–501.
59. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45:D777–D783.
60. **Mukewar SS**, **Sharma A**, Phillip N, et al. Risk of pancreatic cancer in patients with pancreatic cysts and family history of pancreatic cancer. Clin Gastroenterol Hepatol 2018;16:1123–1130.
61. Canto MI, Hruban RH, Fishman EK, et al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology 2012;142:796–804.
62. Vasen H, Ibrahim I, Ponce CG, et al. Benefit of surveillance for pancreatic cancer in high-risk individuals: outcome of long-term prospective follow-up studies from three European expert centers. J Clin Oncol 2016; 34:2010–2019.

Author names in bold designate shared co-first authorship.

Received October 11, 2018. Accepted January 23, 2019.

Reprint requests
Address requests for reprints to: Nicholas J. Roberts, VetMB, PhD, The Johns Hopkins University School of Medicine, Department of Pathology, 1550 Orleans Street, Room 342, Baltimore, Maryland 21287. e-mail: nrobert8@jhmi.edu.

Acknowledgments
Author contributions: Michael Skaro and Nicholas J. Roberts planned and designed study. Michael Skaro, Neha Nanda, Christian Gauthier, Matthäus Felsenstein, Zhengdon Jiang, Miaozhen Qiu, Koji Shindo, Jun Yu, Danielle Hutchings, Ammar Javed, Ross Beckman, Jin He, Christopher L. Wolfgang, Elizabeth Thompson, Ralph H. Hruban, Alison P. Klein, Michael Goggins, Laura D. Wood, and Nicholas J. Roberts collected samples and clinicopathologic data. Michael Skaro, Neha Nanda, Christian Gauthier, Matthäus Felsenstein, Zhengdon Jiang, and Nicholas J. Roberts conducted experiments and generated sequence data. Michael Skaro, Christian Gauthier, Miaozhen Qiu, and Nicholas J. Roberts analyzed data. Michael Skaro and Nicholas J. Roberts wrote the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by the Sol Goldman Pancreatic Cancer Research Center, Susan Wojcicki and Dennis Troper, the Lustgarten Foundation, the Rolfe Pancreatic Cancer Foundation, the Joseph C. Monastra Foundation, the Gerald O. Mann Charitable Foundation (Harriet and Allan Wulfstat, Trustees), and the National Institutes of Health/National Cancer Institute (R00 CA190889 and P50 CA62924).