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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], **Koji Shindo**[1], **Jun Yu**[2,4], **Danielle Hutchings**[1], **Ammar A. Javed**[4], **Ross Beckman**[4], **Jin He**[4], **Christopher L. Wolfgang**[1,2,4,5], **Elizabeth Thompson**[1,2], **Ralph H. Hruban**[1,2,5], **Alison P. Klein**[1,2,5,6], **Michael Goggins**[1,2,5,7], **Laura D. Wood**[1,2,5], and **Nicholas J. Roberts**[1,2,5]

[1]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

[2]The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

[3]Department of Medical Oncology, Sun Yat-Sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, 651 Dongfeng Road East, Guangzhou 510060, China.

[4]Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

[5]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

[6]Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, 21205, USA.

[7]Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

Abstract

Background & aims—Many patients with pancreatic adenocarcinoma (PDAC) carry germline mutations associated with increased risk of cancer. It is not clear whether patients with intraductal

Correspondence: Nicholas J. Roberts, Vet.M.B., Ph.D., The Johns Hopkins University School of Medicine, Department of Pathology, 1550 Orleans Street, Baltimore, MD 21287. Tel: 410-502-5162. Email: nrobert8@jhmi.edu.

Contributions

M.S. and N.J.R planned and designed study. M.S., N.N., C.G., M.F., Z.J., M.Q., K.S., J.Y., D.H., A.J., R.B., J.H., C.L.W., E.T., R.H.H., A.P.K., M.G., L.D.W., and N.J.R. collected samples and clinicopathologic data. M.S., N.N., C.G., M.F., Z.J., and N.J.R. conducted experiments and generated sequence data. M.S., C.G., M.Q., and N.J.R. analyzed data. M.S. and N.J.R. wrote the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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 non-tumor tissue from 315 patients with surgically resected IPMNs, from 1997 through 2017, and sequenced 94 genes with variants associated with cancer risk. Mutations associated with increased risk of cancer were identified and compared to individuals from the Exome Aggregation Consortium.

Results—We identified 23 patients with a germline mutation associated with cancer risk (7.3%; 95% CI, 4.9%–10.8%). Nine patients had a germline mutation associated with pancreatic cancer susceptibility (2.9% 95% CI, 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 to patients with IPMNs without these mutations ($P<.0320$).

Conclusions—In sequence analyses of 315 patients with surgically resected IPMNs, we found 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 to 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

Introduction

Pancreatic adenocarcinoma (PDAC) is a deadly disease with a 5-year survival rate of just 8 percent[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 non-invasive 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]. 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 impact 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, as many established susceptibility genes predispose to tumors in a number of organs, recommended screening for these extra-pancreatic 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 morphological alterations[18–21]. These alterations are well-defined and result in progression from normal epithelium, to non-invasive precursor lesion, and finally invasive carcinoma[22]. Pre-malignant, non-invasive precursor lesions are of three types, microscopic pancreatic intraepithelial neoplasia and macroscopic intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms[23]. As IPMNs are macroscopic and non-invasive, 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 IPMN patients with a high-risk of progression to PDAC and 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 intraluminal spread of neoplastic cells, to an environmental exposure, or 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 IPMN[33–35]. Third, in one screening study of 78 patients at high-risk of pancreatic cancer, most of the patients who underwent pancreatic resection for concerning imaging findings had IPMN[36]. And in another study, the prevalence of incipient and high-grade IPMN was higher in patients with familial compared to 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 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. 350 unselected patients with surgically resected IPMN and available non-tumor tissue were identified from surgical and pathology databases. Where available, 25 mg of fresh-frozen non-tumor tissue (duodenum) was obtained. Otherwise, 0.6 mm tissue cores were obtained from formalin-fixed blocks (FFPE) of non-tumor tissue (duodenum, gallbladder, liver, or spleen).

DNA extraction

DNA was extracted from fresh-frozen non-tumor tissue using the DNeasy Blood & Tissue Kit (Qiagen, catalog no. 69504) according to the manufacturer's instructions. DNA from FFPE non-tumor tissue cores was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, catalog no. 56404) and deparaffinization solution (Qiagen, catalog no. 19093) with the following protocol modifications: 1) 10 or fewer tissue cores were de-paraffinized with 120 μL of deparaffinization solution, while 11 or more tissue cores were deparaffinized with 200 μL of deparaffinization solution, 2) after 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) using the Qubit 1× dsDNA BR Assay Kit (Thermo Fisher Scientific, catalog no. Q32853).

Library preparation, sequencing, and analysis

DNA sequence libraries for each sample were prepared with the TruSight Rapid Capture Kit (Illumina, catalog no. FC-140–1105) and pooled into groups of 12 before capture with the TruSight Cancer probe set (Illumina, catalog no. FC-140–1101) 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 (Agilent, catalog no. G2939BA) using the High Sensitivity DNA Kit (Agilent, catalog no. 5067–4626) and the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) using the Qubit 1× dsDNA HS Assay Kit (Thermo Fisher Scientific, catalog no. Q33230). Captured sequence libraries were further pooled into groups of 24 samples and sequenced on the Illumina MiSeq System (Illumina, CA) using the MiSeq Reagent Kit v2 (300-cycles) (Illumina, catalog no. MS-102–2002), generating 150 base pair (bp) paired-end reads. Sequence reads were processed through a standardized pipeline using MiSeq Reporter Software v2.6 (Illumina, CA). Sequence reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner (BWA)[42]. Variant calling was performed with Genome Analysis Tool Kit (GATK)[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 RefSeq transcripts, minor allele frequency (MAF) using publicly available variant databases (1000 Genomes Project, Exome Variant Server, and Exome Aggregation Consortium (ExAC)), and ClinVar annotations[44–46]. Variants (single base substitutions (SBS) or insertions/deletions (INDEL)) 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 using the Integrative Genomics Viewer[47].

Variant validation

Putative deleterious germline mutations were validated via PCR amplification and Sanger sequencing of the variant region. Primers (Integrated DNA Technologies, Inc., CA) used for amplification are given in Supplementary Table 2. PCR set-up was conducted with OneTaq (NEB, catalog no. M0480S) according to manufacturer's instructions. Amplification was conducted with the T100 Thermo Cycler (BioRad, catalog no. 1861096) using the following cycling conditions: one 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 (Qiagen, catalog no. 28104) and Sanger sequenced (Genewiz, MD). Sequence chromatograms were visualized with 4Peaks (Nucleobytes, Netherlands)

Statistical analysis

Statistical analyses were conducted with Prism 6 (GraphPad Software). Confidence intervals for percent of samples with a hereditary cancer predisposition gene or pancreatic cancer susceptibility gene were calculated using the modified Wald method. Germline mutations in surgically resected IPMN patients and non-TCGA samples from ExAC were grouped by gene and compared using a two-tailed, chi-square test with Yates' correction. Bonferroni correction for multiple testing was used and a P value $< 5.3\times10^{-4}$ was considered significant. Germline mutations in patients with surgically resected IPMN and unselected PDAC patients were grouped by gene and compared using a two-tailed Fisher's exact test. Clinicopathologic variables in surgically resected IPMN patients by presence of germline mutation and invasive cancer were compared using a two-tailed Fisher's exact test, except for age at time at surgery, duration of follow-up, and mean longest diameter of IPMN, which were compared using a two-tailed, unpaired t test. P values < 0.05 were considered significant. P values less than 0.0001 were abbreviated to < 0.0001.

Results

350 patients with surgically resected IPMN were included in this study. 315 patients had greater than 20× average target coverage after sequencing and were included in subsequent analyses. 138 patients had a high-grade IPMN (43.8%), 152 patients had a low- or intermediate-grade IPMN (48.3%), while 25 did not have a reported grade (7.9%). 62 (19.7%) patients had multifocal IPMN. 72 patients had 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). 40 patients (12.7%) had a family history of pancreatic cancer in either a 1st or 2nd 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 Mbp per sample (range: 10–562 Mbp; standard deviation: 138 Mbp). Mean target coverage was 256× (range: 20–877×; standard deviation: 140×). Mean target region covered at 1× and 10× was 99.1% (73.9–100%,

standard deviation: 2.0%) and 97.2% (range: 46.9–100%; standard deviation: 5.6%) respectively. Mean number of SNVs identified per patient was 276 (range: 56–340; standard deviation: 40) and 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 percent confidence interval 4.9–10.8%) (Table 2). 10 germline mutations in 9 patients were in established pancreatic cancer susceptibility genes (2.9%: 95 percent confidence interval 1.3–5.4%), including five germline mutations in *ATM*, three germline mutations in *BRCA2*, one germline mutation in *MSH6*, and one germline mutation in *PALB2*. One germline mutation was also identified in *BUB1B*, a previously identified candidate pancreatic cancer susceptibility gene[11]. More than one patient had a germline mutation involving *ATM* (5 patients), *BRCA2* (3 patients), *FANCI* (2 patients), and *PTCH1* (2 patients). Three patients had more than one germline mutation in a hereditary cancer predisposition gene. One patient had both a *RB1* and *PTCH1* germline mutation, one patient had both a *BRCA2* and *FANCM* 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 considering all sequenced hereditary cancer predisposition genes (*P* value = 0.6590) or pancreatic cancer susceptibility genes (*P* value = 0.1403). Similarly, the majority of individual genes sequenced were not significantly enriched in patients with an IPMN. However, three genes were significantly enriched after Bonferroni correction for multiple testing. These genes are *ATM* (*P* value = < 0.0001), *PTCH1* (*P* value = < 0.0001), and *SUFU* (*P* value = < 0.0001).

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 over- or under-representation in surgically resected IPMN patients compared to unselected PDAC patients.

The patients with IPMN that 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 to 67 of 306 patients without a germline mutation (Fisher's exact test; *p*-value = 0.0320) (Table 4). Interestingly, there was no statistically significant association between a germline mutation in a hereditary cancer predisposition gene and concurrent invasive carcinoma (Table 4). Of the five patients with a germline mutation in a pancreatic cancer susceptibility gene and invasive carcinoma, only one had a family history of pancreatic cancer in a 1st or 2nd 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 to IPMN patients without a germline mutation with respect to family history of pancreatic cancer in 1st or 2nd 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* value = < 0.0001) and involvement of the main pancreatic duct (*P* value = < 0.0059) compared to 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).

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 2.9% had a germline mutation in an established pancreatic cancer susceptibility gene. The number of patients with a germline mutation in a either a hereditary cancer predisposition gene or a pancreatic cancer susceptibility gene was not significant when compared to ExAC controls. However, prevalence of a germline mutation in pancreatic cancer susceptibility genes in IPMN patients is similar to recent studies of PDAC patients unselected for family history where between 3.9 and 5.5% patients had a germline mutation[8, 9].

Three individual genes were significantly enriched in surgically resected IPMN patients compared to ExAC controls. These genes include *ATM* (five germline mutations), *PTCH1* (two germline mutations), and *SUFU* (one 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, while *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 as aberrant Hedgehog signaling has been implicated in pancreatic tumor development. Specifically, over-expression of SHH is observed in over 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.

Interestingly, surgically resected IPMN patients 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 extra-pancreatic 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. While this assured 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 have already or in the future develop PDAC. Assessment of unselected patients is necessary to determine the clinical utility of stratification by germline mutation status in patients with IPMN that have not yet undergone surgical resection. Second, while 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 as a large dataset of similarly sequenced controls was not available. Variant data from ExAC samples was similarly annotated and analyzed to IPMN cases, however, sequencing methodology was different, and this may result in batch effects that hinder analysis of gene associations. Fourth, only limited clinicopathologic 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

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATM	Ataxia telangiectasia mutated
bp	Base pair
BRCA1	breast cancer 1
BRCA2	breast cancer 2
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B
CDKN2A	cyclin-dependent kinase inhibitor 2A
CPA1	carboxypeptidase A1
ExAC	Exome Aggregation Consortium
FANCI	FA complementation group I
FANCM	FA complementation group M
FFPE	formalin fixed, paraffin-embedded
GLI1	GLI family zinc finger 1
IPMNs	intraductal papillary mucinous neoplasms
MAF	minor allele frequency
MLH1	mutL homolog 1
MSH2	mutS homolog 2
PDAC	pancreatic adenocarcinoma
PALB2	partner and localizer of BRCA2
PMS2	PMS1 homolog 2, mismatch repair system component

PARP-1	poly [ADP-ribose] polymerase-1
PRSS1	serine protease 1
PTCH1	patched 1
STK11	serine/threonine kinase 11
SUFU	SUFU negative regulator of hedgehog signaling

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Table 1.

Demographics and characteristics of patients with surgically resected IPMN

Characteristic[1]		Number	Percent
Race			
	White	270	85.7
	Other	45	14.3
Sex			
	Male	162	51.4
	Female	153	48.6
Age			
	<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			
	<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 IPMN			
	1	253	80.3

Characteristic[1]		Number	Percent
	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

[1] IPMN - intraductal papillary mucinous neoplasm. NR - not reported. Family history of pancreatic cancer in 1st and 2nd degree relatives.

Table 2.

Germline mutations identified in patients with surgically resected IPMN

Patient number	Gene	Type	Transcript	Germline mutation[1]			Functional consequence	Concurrent invasive carcinoma
1	*ATM*		NM 000051	g.chr11:108098600 G>A	c.G170A	p.W57X	Stopgain	Signet ring carcinoma
2	*ATM*		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*	Pancreatic	NM 000051	g.chr11:108175549 C>T	c.C5644T	p.R1882X	Stopgain	PDAC
5	*ATM*	cancer	NM 000051	g.chr11:108206686 A>T	c.A8266T	p.K2756X	Stopgain	-
6	*BRCA2*	susceptibility	NM 000059	g.chr13:32907014 A>T	c.A1399T	p.K467X	Stopgain	-
7	*BRCA2*	gene	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	NM 004304	g.chr2:29436851 G>A	c.C3742T	p.R1248X	Stopgain	-
11	*BRIP1*	cancer	NM 032043	g.chr17:59871059 C>A	c.G1372T	p.E458X	Stopgain	Adenosquamous PDAC
12	*BUB1B*	susceptibility	NM 001211	g.chr15:40462282 C>T	c.C199T	p.R67X	Stopgain	PDAC
13	*CDH1*	gene	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	-

IPMN - Intraductal papillary mucinous neoplasm, PDAC - pancreatic adenocarcinoma.

[1] g - genomic change, c - transcript change; p - protein change associated with germline mutation. Genomic co-ordinates use hg19 version of human genome.

Table 3.

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

Germline mutation	IPMN			EXAC			
	AC	AN	AF	AC	AN	AF	*P* value
Hereditary cancer gene	26	630	0.041	3921	105586	0.037	0.6590
Pancreatic cancer susceptibility gene	10	630	0.016	992	105732	0.009	0.1403
ATM	5	630	0.008	134	106203	0.001	<0.0001 *
BRCA2	3	630	0.005	216	106188	0.002	0.2858
MSH6	1	630	0.002	261	106196	0.002	0.9709
PALB2	1	630	0.002	63	106206	0.001	0.8413
ALK	1	630	0.002	24	106209	0.000	0.3570
BRIP1	1	630	0.002	120	106202	0.001	0.7336
BUB1B	1	630	0.002	32	106209	0.000	0.4874
CDH1	1	630	0.002	9	96677	0.000	0.0861
FANCA	1	630	0.002	117	105585	0.001	0.7189
FANCD2	1	630	0.002	83	106209	0.001	0.9947
FANCI	2	630	0.003	83	106208	0.001	0.1569
FANCM	1	630	0.002	174	106183	0.002	0.9746
NBN	1	630	0.002	59	103676	0.001	0.7286
PTCH1	2	630	0.003	14	105834	0.000	<0.0001 *
RB1	1	630	0.002	6	106198	0.000	0.0235
RECQL4	1	630	0.002	173	105674	0.002	0.9754
SUFU	1	630	0.002	0	105586	0.000	<0.0001 *
WT1	1	630	0.002	13	105241	0.000	0.1476

IPMN - intraductal papillary mucinous neoplasm; ExAC - Exome Aggregation Consortium; AC - germline mutation allele count, AN - assessed allele number; AF - frequency of germline mutations.

* 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

Variable[1]	Germline mutation in hereditary cancer predisposition gene			Germline mutation in pancreatic cancer susceptibility gene		
	+ (n=23)	– (n=292)	p-value	+ (n=9)	– (n=306)	*P* value
Patients with concurrent invasive carcinoma (n)	9	63	0.0694	5	67	0.0320
Patients with family history of pancreatic cancer (n)	6	34	0.0971	3	37	0.1670
Patients with personal history of cancer (n)	1	53	0.1419	1	53	1.0000
Mean age at surgery (years)	65.2	68.2	0.1911	62.2	68.2	0.1025
Male patients (n)	14	148	0.3916	6	156	0.5031
Patients with high-grade dysplasia (n)	8	130	0.6442	2	136	0.6865
Mean longest diameter of IPMN (cm)	2.1	2.7	0.0986	2.1	2.7	0.3674
Patients with multifocal IPMN (n)	4	58	1.0000	2	60	0.6921
Patients with main duct involvement (n)	6	106	1.0000	2	110	0.3078
Mean duration of follow-up (months)	46.8	32.5	0.1248	40.2	33.2	0.6287
Incident pancreatic cancer during follow-up (n)	0	2	1.0000	0	2	1.0000

[1]Not all patients had a grade of dysplasia assigned. P-values calculated 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).