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Mutant TP53 in Duodenal Samples of Pancreatic Juice from Patients with Pancreatic Cancer or High-Grade Dysplasia

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Abstract

Background & Aims—Imaging tests can identify patients with pancreatic neoplastic cysts but not microscopic dysplasia. We investigated whether mutant *TP53* can be detected in duodenal samples of secretin-stimulated pancreatic juice, and whether this assay can be used to screen for high-grade dysplasia and invasive pancreatic cancer.

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Author contributions:

Mitsuro Kanda; acquisition of data; analysis and interpretation of data; drafting of the manuscript. Yoshihiko Sadakari, Spencer Knight; Michael Borges; Sho Fujiwara; acquisition of data

Mark Topazian, Sapna Syngal, James Farrell, Jeffrey Lee, Ihab Kamel, Anne Marie Lennon; generation of data, revision of the manuscript

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Michael Goggins; study concept and design; study supervision; interpretation of data; drafting of the manuscript; obtained funding

Methods—We determined the prevalence of mutant *TP53* in microdissected pancreatic intraepithelial neoplasias (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and invasive adenocarcinomas. *TP53* mutations were quantified by digital high-resolution melt-curve analysis and sequencing of secretin-stimulated pancreatic juice samples, collected from duodena of 180 subjects enrolled in Cancer of the Pancreas Screening trials; patients were enrolled because of familial and/or inherited predisposition to pancreatic cancer, or as controls.

Results—*TP53* mutations were identified in 9.1% of intermediate-grade IPMNs (2/22), 17.8% of PanIN-2 (8/45), 38.1% of high-grade IPMNs (8/21), 47.6% of PanIN-3(10/21), and 75% of invasive pancreatic adenocarcinomas (15/20); no *TP53* mutations were found in PanIN-1 lesions or low-grade IPMNs. *TP53* mutations were detected in duodenal samples of pancreatic juice from 29/43 patients with pancreatic ductal adenocarcinoma (67.4% sensitivity; 95% confidence interval, 0.52–0.80) and 4/8 patients with high-grade lesions (PanIN-3 and high-grade IPMN). No *TP53* mutations were identified in samples from 58 controls or 55 screened individuals without evidence of advanced lesions.

Conclusion—We detected mutant *TP53* in secretin-stimulated pancreatic juice samples collected from duodena of patients with high-grade dysplasia or invasive pancreatic cancer. Tests for mutant *TP53* might be developed to improve the diagnosis of and screening for pancreatic cancer and high-grade dysplasia.

clinicaltrials.gov (NCT00438906, NCT00714701)

Keywords

Pancreatic cancer; pancreatic intraepithelial neoplasm; intraductal papillary mucinous neoplasm; pancreatic juice; *TP53*; mutation; secretin; tumor; biomarker; diagnostic; early detection

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States¹. Most patients with pancreatic ductal carcinoma are diagnosed with advanced disease. Patients who present with early-stage disease have the best outcome, but surgery is only an option for ~15% of patients with pancreatic cancer¹. Early detection is considered potentially one of the most effective approaches to improving the prognosis of this dismal disease.

Effective early detection necessitates detecting potentially curable lesions before they become symptomatic such as subcentimeter invasive cancers and precursors with high-grade dysplasia². The commonest precursors to pancreatic adenocarcinoma are PanINs. PanINs are microscopic lesions (by definition <5mm diameter); IPMNs are larger cystic pancreatic precursor neoplasms³ with an estimated prevalence of ~2% of older adults⁴. PanINs and IPMNs are generally asymptomatic and are only identified incidentally or by screening.

Screening is justifiable only when offered to individuals at sufficient risk of developing pancreatic cancer and when the screening test is safe and effective. An ideal screening test would be a highly accurate blood test; however, no blood test has been shown to be as accurate as pancreatic imaging tests (CT, EUS) for diagnosing symptomatic pancreatic cancers, never mind small asymptomatic cancers and precursor neoplasms.

Screening protocols for individuals with a strong family history of pancreatic cancer utilize pancreatic imaging tests (usually EUS and/or MRI/MRCP)^{5–12}. Individuals at significantly increased risk can be identified based on their family history of the disease, but we still lack an effective screening test to offer these individuals¹³. EUS and MRI/MRCP are very

effective for identifying small pancreatic cysts⁵, but PanINs are generally too small to be identified by these tests¹⁴; they are only identified after histological examination of resected pancreata. Low-grade PanIN-1 lesions are prevalent in older adults but PanIN-3 lesions (high-grade dysplasia) are usually found in pancreata of patients with invasive pancreatic cancer and in subjects undergoing pancreatic screening^{15, 16}. The inability to identify PanINs preoperatively highlights the need for novel diagnostic approaches to identify PanINs. One promising approach is to analyze pancreatic juice for mutations arising from pancreatic neoplasms. Markers of pancreatic cancer in ductal pancreatic juice collected during ERCP has been studied^{17, 18}, but ERCP is too invasive to employ for pancreatic screening. We recently reported on the diagnostic potential of secretin-stimulated pancreatic juice samples collected from the duodenum during upper endoscopy¹⁹. We found that *GNAS* mutations, a highly specific marker of IPMNs, were reliably detected in these samples in subjects with IPMNs and diminutive cysts (<5mm), suggesting that pancreatic juice is a reliable sample for detecting molecular alterations in the pancreatic ductal system¹⁹.

Useful markers of pancreatic neoplasia need to accurately distinguish early invasive pancreatic cancers and high-grade dysplasia (PanIN-3 and IPMNs with high-grade dysplasia) from lesions with low-grade dysplasia. Mutant *TP53* may be one such marker. It is mutated in ~75% of invasive pancreatic cancers^{20, 21}, with a similar prevalence in familial and sporadic pancreatic cancers^{21, 22}, and immunohistochemical studies suggest *TP53* mutations occur late in the progression of PanIN lesions²³. In contrast, other genes commonly mutated in pancreatic ductal adenocarcinomas, *KRAS*, *p16* and *SMAD4*, do not have these diagnostic characteristics: *KRAS* mutations are present in >90% of PanIN-1 lesions²⁴, and are commonly detected in the pancreatic juice of controls^{18, 24}. *P16* mutations are thought to also arise throughout PanIN development so these mutations may not distinguish low-grade from high-grade PanINs. Genetic inactivation of *SMAD4* is thought to be specific for PanIN-3 and invasive cancer²⁵, but *SMAD4* is commonly inactivated by homozygous deletion and such alterations have only been detected in secondary fluids when the deletion has been first characterized in the primary cancer²⁶.

In this study, we determined the prevalence of *TP53* mutations in PanIN and IPMNs, and used digital high-resolution melt-curve analysis (digital-HRM) and sequencing to measure *TP53* mutation concentrations in duodenal collections of pancreatic juice of individuals undergoing pancreatic evaluation.

MATERIALS AND METHODS

All elements of this investigation have been approved by The Johns Hopkins Medical Institutional Review Board and written informed consent was obtained from all patients. All authors had access to the study data and had reviewed and approved the final manuscript.

Patients and specimens

Pancreatic juice samples and subject data for this study were obtained from 180 participants enrolled in the CAPS2, CAPS3 and CAPS4 clinical trials^{5, 9} (clinicaltrials.gov (NCT00438906, NCT00714701)). Subjects enrolled for screening were asymptomatic with either (i) a strong family history of pancreatic cancer (at least 2 affected blood relatives with pancreatic cancer related by first-degree); the eligibility age in CAPS4 was 50–80 years or 10 years younger than the youngest pancreatic cancer in the kindred, (ii) germline mutation carriers (*BRCA2*, *p16*, *BRCA1*, HNPCC genes) with a family history of pancreatic cancer or (iii) Peutz-Jeghers syndrome. Disease controls undergoing pancreatic evaluation were also enrolled to evaluate pancreatic juice markers, and we included subjects from CAPS2–4

to have sufficient disease controls and adequate follow-up subsequent to pancreatic juice collections to identify prospective cancers. See Supplemental Methods for further details.

Pancreatic juice secretion was stimulated by infusing intravenous human synthetic secretin (0.2 ug/kg over one minute); 10–20mls of juice was then collected from the duodenal lumen for ~5 minutes by suctioning fluid through the echoendoscope channel. Secretin was provided for CAPS3 and CAPS4 (ChiRhoClin Inc, Burtonsville, Maryland), and CAPS2 (Repligen Corp., Mass). Juice aliquots (10–20) were stored without additional processing at –80°C prior to use. Extracted DNA was quantified by real-time PCR¹⁹.

Laser capture microdissection

PanINs, IPMNs and normal duct samples identified during intra-operative frozen section analysis of resected pancreata by RHH (from 2007–2010) were selected for *TP53* analysis as previously described²⁴. For comparison, we analyzed frozen sections of pancreatic ductal adenocarcinomas (see table 1 and Supplemental Methods).

High-resolution melt-curve analysis

To evaluate DNA from PanIN, IPMN and ductal adenocarcinoma tissues for mutations, HRM was performed in triplicate as previously described²⁴. We evaluated the limit of detection and accuracy of digital-HRM (Figure S1).

For juice analysis, digital-HRM analysis was employed and assays were almost always performed blinded to the final diagnosis. In each 96-well plate, 900 genome equivalents of pancreatic juice DNA were dispensed into 90 wells (10 genome equivalents per well); five wells had wild-type DNA, one had water. Juice DNA was consistently amplified (88–90 wells); no juice samples were excluded for poor DNA quality. Primers and PCR conditions are listed in Table S1. *TP53* exons 5–8 were analyzed (almost all *TP53* mutations occur in these exons). Melt-curve analysis was performed as described²⁴.

Sanger sequencing and pyrosequencing

PCR products were used as templates for Sanger sequencing and mutations identified in PCR products by digital-HRM and Sanger were confirmed by pyrosequencing²⁴. Sequencing was performed on at least three representative digital-HRM-positive wells per juice sample, and one HRM-wild-type and HRM-negative control well. To ensure assay specificity, a juice sample was only deemed as having a mutation when the same mutation was identified in two or more PCRs by both Sanger and pyrosequencing. The number of mutation-positive PCR products confirmed by sequencing was defined as “the mutation score” for a juice sample.

We next analyzed pancreatic juice samples collected from the operating room of five patients with known *TP53* mutations in the primary pancreatic cancer. In all cases, the same *TP53* mutations of the cancer were detected in the juice samples by Sanger sequencing of HRM-positive wells (Figure S2, Table S2).

Statistical analysis

Median mutation scores between patient groups were compared by Mann-Whitney’s U test. Chisquare was used to compare associations between *TP53* mutational status and clinical factors. ANOVA was used to evaluate associations between clinical factors and positive mutation scores. Statistical analysis was performed using SPSS 17.0 software (SPSS, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Prevalence of TP53 mutations in PanINs, IPMNs and ductal adenocarcinomas

TP53 mutations were detected in 9.1% of intermediate-grade IPMNs, 17.8% of intermediate-grade PanINs (PanIN-2), 38.1% of high-grade IPMNs, 47.6% of PanIN-3, and 75% of 20 invasive ductal adenocarcinomas (one cancer had two mutations)(Table 1). No *TP53* mutations were detected in low-grade PanINs (PanIN-1) or IPMNs.

Detection of mutant *TP53* in duodenal collections of pancreatic juice

We first evaluated the limit-of-detection of our digital-HRM assay. Mutant *TP53* could reliably detect 0.1–10% concentrations of mutant-to-wild-type DNA (Figure S1).

We next employed digital-HRM to detect *TP53* mutations in duodenal collections of secretin-stimulated pancreatic juice samples during EUS from CAPS study subjects. *TP53* mutations were detected by digital-HRM in secretin-stimulated pancreatic juice samples of 29/43 patients with pancreatic cancer (sensitivity 67.4%, Figure 1A). For all juice samples, *TP53* mutations identified in HRM-positive PCR wells were confirmed by both Sanger and pyrosequencing. Two individuals with pancreatic cancer had 2 different *TP53* mutations identified in their juice sample (see Figure 1 for representative results). There was no significant association between *TP53* status and clinicopathological factors or outcome. Larger tumor size (≥3cm vs. <3cm) was associated with higher juice mutation score ($P=0.0354$, Table S3), but there was no significant correlation overall between tumor size and mutation score ($r=0.16$, $p=0.065$).

No *TP53* mutations were detected in juice samples of any subjects with normal pancreata (including subjects undergoing screening) ($n=64$), or with chronic pancreatitis ($n=24$)(Figure 1A).

Detection of mutant *TP53* in pancreatic juice prior to a pancreatic cancer diagnosis

One individual with pancreatic cancer had been enrolled in CAPS4 for screening purposes. He had undergone baseline screening 13 months prior to his pancreatic cancer diagnosis. At baseline screening, he was asymptomatic with no mass either by EUS or MRI/MRCP. The only detected abnormalities were two subcentimeter cysts in the pancreatic head and body. There was no change found at follow-up EUS 6 months prior to diagnosis. At his next surveillance visit, EUS and MRI/MRCP again found the cysts were stable, but a new mass lesion was identified in the pancreatic tail and FNA confirmed pancreatic ductal adenocarcinoma. A distal pancreatectomy was performed. His pancreatic juice collected from the duodenum during baseline EUS (analyzed after the cancer diagnosis) contained a *TP53* mutation (exon 7 13318G>A, R248Q, mutation score 4). This *TP53* mutation was also present in his endoscopic juice sample obtained at his diagnostic EUS (mutation score 17), and in his primary pancreatic cancer. He did not have juice collected 6 months prior to diagnosis.

We know of no other pancreatic cancers that have developed in the screening group since their juice analysis (median follow-up, 36.4/15 months, range, 6.2–49.3 months).

Pancreatic juice *TP53* mutations in subjects with precursor lesions

We next examined secretin-stimulated pancreatic juice samples from 49 individuals with evidence of pancreatic precursor neoplasms. These individuals had undergone pancreatic evaluation either for screening ($n=25$), or for suspected IPMN identified incidentally ($n=24$) and included subjects who underwent pancreatic resection after pancreatic evaluation ($n=28$) (see Table 3) to enable us to compare pancreatic juice results to the pathology of the

resected pancreata. *TP53* mutations were detected in endoscopic duodenal collections of pancreatic juice of four (50%) of 8 individuals whose most advanced pathology was high-grade dysplasia (PanIN-3 and/or high-grade IPMN) and in one (6.7%) of 15 individuals whose highest grade pathology was intermediate-grade dysplasia (PanIN-2 or in IPMN), and in none of 5 individuals with only low-grade dysplasia, or in any of 21 subjects undergoing surveillance for suspected IPMNs (Figure 1A)(median (largest) cyst size, 14.0 (5–28 mm), median number, 2.8 (range 1–8).

The mutant *TP53* mutation score in pancreatic juice was significantly lower in subjects without invasive cancer than in those who had invasive pancreatic cancer ($P=0.0191$, Figure 2A). Representative examples of melt-curves and sequencing are shown in Figure 2B).

DISCUSSION

Our results indicate that mutant *TP53* detected in duodenal collections of secretin-stimulated pancreatic juice may provide evidence that the pancreas contains either microscopic PanIN-3 IPMN with high-grade dysplasia, or invasive pancreatic ductal adenocarcinoma. Mutant *TP53* was detected in the pancreatic juice of only 1 of 102 individuals not known to have high-grade dysplasia, and that was a patient with a 6cm IPMN with intermediate-grade dysplasia. The prevalence of mutant *TP53* detected in the duodenal juice collections of patients with pancreatic cancers (67.4%) is similar to its prevalence in primary resected pancreatic cancers (~75%)^{24, 25} which suggests that mutant DNA arising from a patient's pancreatic cancer can generally be measured in their duodenal collections of pancreatic juice.

Although *TP53* mutation concentrations were significantly higher in subjects with larger compared to smaller cancers and in those with PanIN-3/IPMN with high-grade dysplasia, there was no correlation between tumor size and juice mutation concentrations. This is not surprising. Juice mutation concentrations are based on normal DNA concentrations which probably vary considerably in juice. Pancreatic cancer tumor cellularity also varies considerably; hence tumor size provides only approximate estimates of cancer cell numbers (and DNA). Finally, ductal obstruction may influence tumor DNA amounts released into pancreatic juice.

Importantly, *TP53* mutations were not detected in the juice samples of any of the patients in the disease control groups ($n=54$), screened individuals with normal pancreata ($n=30$) or in individuals who were undergoing surveillance for small pancreatic cysts identified incidentally or through screening ($n=21$). Patient samples analyzed in this study were representative of our CAPS study population and included subjects who had subtle EUS changes suggestive of PanIN¹⁶. Although we did not identify *TP53* mutations in subjects with chronic pancreatitis, prior studies using older technologies have reported finding *TP53* mutations in ERCP-collected juice samples of a small percentage (<10%) of individuals with chronic pancreatitis^{18,19,31–33}. These older studies did not employ the more accurate DNA sequencing methods used in this study to identify mutations, therefore additional studies are needed in order to have better estimates of the prevalence of *TP53* mutations in juice samples from patients with chronic pancreatitis^{17, 18, 27–29}.

Since an important goal of pancreatic juice research is to determine if biomarkers could be used to accurately find high-grade dysplasia or small invasive cancers not visible by pancreatic imaging tests, the results in patients with evidence of high-grade dysplasia are informative. In our study, mutant *TP53* was detected in the juice samples of 4 of 8 individuals whose highest grade lesion was high-grade dysplasia, including 2 of 5 individuals whose highest lesion was a PanIN-3. One of these two individuals also had an

IPMN with low-grade dysplasia. Since we did not detect mutant *TP53* in any IPMNs with low-grade dysplasia, the pancreatic juice *TP53* mutation detected in this patient likely arose from their PanIN-3 lesion. Since PanINs are not typically detectable by pancreatic imaging tests, the ability of *TP53* measurements to find evidence of microscopic high-grade dysplasia highlights the potential of pancreatic juice analysis to complement pancreatic imaging for individuals undergoing screening.

Our study also showed that the detection rate of mutant *TP53* in the pancreatic juice of patients with precursor neoplasms is concordant with the presence of mutant *TP53* we identified in resected PanIN and IPMN specimens. *TP53* mutations were only detected in PanINs and IPMNs with intermediate-grade (15%) and high-grade dysplasia (43%), not in any low-grade IPMNs or PanIN-1. These figures are consistent with prior estimates of *TP53* mutation in these lesions determined from immunohistochemical analysis (reviewed in³).

Mutant *TP53* was also detected in the juice of 2 of 3 individuals whose highest grade lesion was an IPMN with high-grade dysplasia. This indicates that pancreatic juice analysis has the potential to be useful for identifying high-grade dysplasia within IPMNs. Although clinical guidelines for resecting IPMNs are very helpful for managing asymptomatic patients with pancreatic cysts³⁰, there is a need to determine if molecular markers can be used along with current clinical guidelines to improve the selection of patients needing resection of their IPMN.

A suspected IPMN is the most common indication for pancreatic resection among high-risk individuals undergoing pancreatic screening^{5–11}. In our cohort, concern for undetected PanIN-3 lesions and early invasive cancer was an important consideration when surgery was recommended for patients with lesions identified by screening. Hence, lesions identified in high-risk individuals are often resected before they reach the modified Tanaka criteria used as resection criteria for sporadic IPMNs³⁰.

The limitations of current pancreatic imaging tests were highlighted in the one patient in this study who was diagnosed with pancreatic cancer 13 months after his baseline pancreatic screening. Based on our current understanding of the natural history of early invasive pancreatic cancer³¹, it is likely this patient had a high-grade precursor or an invasive pancreatic cancer at the time he was initially screened that was below the limit of detection of his baseline and follow-up EUS and MRI. This is not surprising. An early pancreatic cancer may not become visible until it forms a solid 5+ mm diameter mass. At this size, a pancreatic cancer can grow and spread rapidly as evidenced by the presence of this patient's lymph node metastases at diagnosis. This case highlights the potential of pancreatic juice *TP53* measurements to herald the subsequent detection of invasive pancreatic cancer.

Until further evidence is available about its diagnostic utility, it is premature to recommend surgical resection based solely on the detection of mutant *TP53* in pancreatic juice. Additional information is needed about the diagnostic accuracy of mutant *TP53* in patients undergoing pancreatic evaluation. If additional investigations confirm that mutant *TP53* detected in pancreatic juice is an accurate predictor of higher-grade dysplasia or invasive pancreatic cancers such a test would likely have clinical utility, particularly in the high-risk population where the prevalence of precursor neoplasms is high⁵.

Future research using markers such as *TP53* may also shed light on the natural history of precursor lesions with high-grade dysplasia in high-risk individuals. Since the detection of pancreatic juice mutant *TP53* could indicate either invasive cancer or intermediate/high-grade dysplasia, particularly for individuals without a suspicious lesion detected by imaging, an important question is whether they should continue to undergo surveillance or be considered for pancreatic resection.

The absence of *TP53* mutations does not mean a patient does not have high-grade dysplasia or invasive pancreatic cancer. Therefore, an ideal pancreatic juice test would include markers capable of identifying *TP53*-wild-type lesions, and like mutant *TP53*, are highly specific for high-grade neoplasia (PanIN-3 and IPMNs with high-grade dysplasia) and early invasive pancreatic cancer.

Some strengths of this study are the large sample size, the multicenter population, including subjects undergoing pancreatic screening, long-term follow-up of patients after screening, and the demonstration that *TP53* mutations can be detected in the duodenal collections of pancreatic juice in individuals with PanIN-3 lesions. One limitation of this study is the difficulty identifying specific PanINs as the source of mutations. Since most patients undergoing screening do not undergo resection, we do not have a comprehensive pathological evaluation of their pancreata to identify all their PanIN lesions. Indeed, even when a patient undergoes partial pancreatectomy, PanIN lesions may be present in their remnant pancreata. This is a challenge for all studies evaluating pancreatic juice markers of PanINs.

In conclusion, we find that *TP53* mutations detected in secretin-stimulated pancreatic juice are a highly specific indicator of the presence of high-grade dysplasia (in IPMNs and/or PanIN-3) or invasive pancreatic ductal adenocarcinomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IPMN	intraductal papillary mucinous neoplasm
PanIN	pancreatic intraepithelial neoplasia
GNAS	Guanine Nucleotide-Binding Protein, Alpha-Stimulating
EUS	endoscopic ultrasonography
CT	computed tomography
MRI	magnetic resonance imaging
MRCP	magnetic resonance cholangiopancreatography
ERCP	endoscopic retrograde cholangiopancreatography
FNA	fine-needle aspiration
HRM	high-resolution melt-curve analysis
PCR	polymerase chain reaction
CAPS	Cancer of the Pancreas Screening

LCM	Laser Capture Microdissection
FDR	first-degree relative
s.d	standard deviation
HNPCC	hereditary non-polyposis colorectal cancer
WT	wild-type

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A Prevalence of TP53 mutation in each diagnostic group

B Exon5, 12402 G>A, A159T

C Exon8, 13732 G>A, V272M

Figure 1.

(A) Prevalence of *TP53* mutation by diagnostic group. (B, C) Shifted melt curves, difference curves, and Sanger sequencing of digital-HRM-positive PCRs from juice samples from subjects with pancreatic cancer; Arrows indicate mutations. (B) Case 40 (C) Case 29 (Table 2).

Figure 2.

(A) Mutant *TP53* juice concentrations (mean mutation score) (Y-axis) from subjects with pancreatic cancer and high-grade precursor lesions (x-axis) * $P=0.0191$. (B) *TP53* mutation detected in a pancreatic juice sample from a subject with PanIN-3 (Case 4, Table 3). Shifted melt curves, difference curves and sequencing of digital-HRM-PCRs. Arrows indicate mutations.

Table 1

Prevalence of TP53 mutations in resected fresh-frozen tissue samples of PanINs, IPMNs and pancreatic ductal adenocarcinomas

Patient #	Pathological grade	N	TP53 mutation					Overall	
			Exon 5	Exon 6	Exon 7	Exon 8			
Normal duct		20	0	0	0	0	0	0 (0%)	
IPMN	Low grade dysplasia	15	0	0	0	0	0	0 (0%)	
	Intermediate grade dysplasia	22	1	12419 G>C; K164N	1	12669 A>G; E221G	0	2 (9.1%)	
	High grade dysplasia	21	3	12430 A>C; H169P 12451 G>A; R175H 12463 A>C; H179P	2	12607 T>A; N200K 12617 G>A; E204K	1	13765 C>T; R283C 13787 G>C; R290P	8 (38.1%)
PanIN	PanIN-1A	50	0	0	0	0	0	0 (0%)	
	PanIN-1B	52	0	0	0	0	0	0 (0%)	
	PanIN-2	45	2	12390 A>C; T155P 12463 A>C; H179P	2	12633 G deletion 12614 G>A; V203M	1	13735 C>T; R273C 13738 G>T; V274F 13779 T deletion	8 (17.8%)
PanIN	PanIN-3	21	3	12375 A>T; T144S 12390 A>C; T155P 12453 T>A; C176S	3	12633 G deletion 12586 T>A; H193Q 12593 C>T; R196stop	2	13736 G>A; R273H 13779 T deletion	10 (47.6%)
	Pancreatic ductal adenocarcinoma	20	12337 T>C; L137P	12375 A>T; T144S	12591 C>T; I195T	13257 G deletion	13721 A>T; N268I	13726 T>A; A270I	
			12402 G>A; A159T	12614 G>A; V203M	13273 A>C; H233P	13735 C>T; R273C	13762 C>T; R282W		
12451 G>A; R175H			12639 C>T; T211I	13317 C>T; R248W	13765 C>T; R283C	13787 G>C; R290P			
			12463 A>G; H179R						13777 G>T; E295stop

Table 2

TP53 analysis of endoscopic duodenal collections of pancreatic juice from 43 subjects with pancreatic cancer

	Gender	Age	Diagnostic method	Tumor location	Tumor size (cm)	Differentiation	UICC stage	TP53 in pancreatic juice		
								Status	Mutation score	Mutation
1	Female	77	Surgery	Head	4	Moderate	T3N1	Mutant	8	6 12628 T>A, D207E
2	Male	72	FNA	Body	4	Moderate	T3N0	Wild type	0	
3	Male	40	Surgery	Head	3	Poor	T3N1	Mutant	36	5 12451 G>A, R175H
4	Female	55	FNA	Head	3	Not recorded	T4N1	Mutant	6	8 13792 A>G, K292E
5	Female	79	FNA	Body	2.5	Well	T4N0	Wild type	0	
6	Female	65	FNA	Head	6	Not recorded	T3N0	Mutant	9	8 13792 C>T, R282W
7	Male	68	Surgery	Head	10	Not recorded	T4N1	Mutant	26	6 12588 T>G, L194R
8	Female	55	Surgery	Head	2	Poor	T3N1	Mutant	5	7 13317 C>T, R248W
9	Male	72	FNA	Uncinate	3.8	Well	T3N0	Mutant	10	5 12390 A>C, T155P
10	Male	55	Surgery	Head	2	Moderate	T3N1	Mutant	5	6 12666 A>G, Y220C
11	Male	58	Surgery	Head	3	Not recorded	T3N0	Wild type	0	
12	Female	68	FNA	Body	1.8	Poor	T1N0	Mutant	3	5 12449 G>T, R174S
13	Female	57	FNA	Tail	6.5	Not recorded	T4N0	Mutant	33 4	6 12647 C>G, H214D 8 13812 G>T, E298D
14	Female	64	FNA	Head	4.1	Not recorded	T3N1	Mutant	7	7 13317 C>T, R248W
15	Female	68	Surgery	Head	2	Moderate	T3N1	Mutant	5	5 12399 C>T, R158C
16	Female	69	Surgery	Body	1.5	Not recorded	T1N0	Wild type	0	
17	Female	63	FNA	Head	2.3	Not recorded	T3N0	Wild type	0	
18	Male	75	FNA	Head	4.5	Not recorded	T3N1	Mutant	4	8 13786 C>T, R290C
19	Female	69	FNA	Tail	3.5	Well	T4N1	Wild type	0	
20	Female	56	FNA	Head	3.5	Not recorded	T3N0	Wild type	0	
21	Male	52	FNA	Head	1.7	Not recorded	T3N0	Wild type	0	
22	Male	67	FNA	Head	3.3	Not recorded	T3N1	Mutant	6	6 12609 T deletion
23	Female	61	Surgery	Head	3	Poor	T2N0	Wild type	0	
24	Female	56	FNA	Body	4.5	Not recorded	T4N1	Wild type	0	
25	Female	55	Surgery	Uncinate	3	Poor	T3N1	Wild type	0	
26	Male	70	FNA	Head	3.1	Not recorded	T2N0	Mutant	5	8 13735 C>T R273C

	Gender	Age	Diagnostic method	Tumor location	Tumor size (cm)	Differentiation	UICC stage	TP53 in pancreatic juice			
								Status	Mutation score	Exon	Mutation
27	Male	61	Surgery	Head	4	Moderate	T3N0	Mutant	8	7	13299 T>A, C242S 13792 C>T, R282W
28	Female	74	Surgery	Head	3.5	Moderate	T3N1	Mutant	19	5	12417 A deletion
29	Male	75	Surgery	Head	3.5	Moderate	T3N1	Mutant	43	8	13732 G>A, V272M
30	Female	61	FNA	Body	2.4	Not recorded	T2N0	Mutant	12	6	12645 G>T, R213L
31	Male	71	Surgery	Head	2.1	Not recorded	T3N0	Mutant	12	5	12436 C>T, T170M
32	Male	76	FNA	Uncinate	4.4	Not recorded	T2N0	Wild type	0		
33	Male	64	FNA	Tail	2.1	Not recorded	T2N0	Wild type	0		
34	Male	64	FNA	Tail	1.8	Not recorded	T1N0	Mutant	7	7	13300 G>A, C242Y
35	Male	80	Surgery	Head	2.5	Poor	T4N1	Mutant	18	7	13276 A>G, Y234C
36	Female	78	Surgery	Head	3.5	Moderate	T2N1	Mutant	25	7	13300 G>A, C242Y
37	Female	59	Surgery	Body	2.5	Moderate	T2N0	Mutant	8	5	12393 C>T, R156C
38	Male	57	Surgery	Tail	2.8	Poor	T2N1	Mutant	4	7	13318 G>A, R248Q
39	Male	53	FNA	Head	3.5	Moderate	T3N0	Mutant	9	6	12662 C>T, P219S
40	Female	73	Surgery	Head	2.5	Not recorded	T2N1	Mutant	5	5	12402 G>A, A159T
41	Female	79	Surgery	Body	2.5	Moderate	T2N0	Mutant	5	7	13312 T>C, M246T
42	Male	73	FNA	Head	2.9	Not recorded	T4N1	Wild type	0		
43	Male	62	FNA	Head	2.5	Not recorded	T2N1	Mutant	11	5	12424 C>T, S166L

Table 3

TP53 mutation analysis of duodenal collections of pancreatic juice of 28 subjects with resected pancreatic precursor neoplasms (IPMNs and PanINs)

Gender / Age, Risk		Preoperative imaging		Surgery	Final pathologic diagnosis		TP53 in pancreatic juice			
CAPS	Contrast MRI or CT	EUS	Procedure / Year	Indication for surgery	Highest grade	Additional information	Status	Mut score	Exon	Mut
1	Female / 77, 2 FDR, 2 CAPS2	CT: No evidence of pancreatic mass	Communicating cyst (head, 9mm), focal echogenic thickened wall (6mm) Dilated MPD; head, 4.7mm, Dx; BD-IPMN	Whipple / 2005	Pancreatic cyst	PanIN3	Multifocal PanIN1–3, BD-IPMN low grade; 6mm	Wild type	0	
2	Female / 72, 1 FDR, 2 SDR, CAPS 2	MRI; Dilated tail MPD	Focally dilated MPD cystic appearing (tail, 11mm), focal hypoechoic thickened wall (4mm), Dx; IPMN	Distal panc / 2004	IPMN MPD	PanIN3	2 BD-IPMN 6, 4mm Multifocal PanINs including several foci of PanIN3	Mutant	4	7
3	Female / 66, 2 FDR, CAPS3	MRI; 2 communicating cysts (body 1.1–1.4cm) Dx: BD-IPMN	6 communicating cysts (5–12mm head, body, tail), 1 cyst with mural nodule, 1 solid mass 7mm, Dx: BD-IPMNs, PNET	Total panc / 2007	Pancreatic cyst	PanIN3	3 BD-IPMN int-grade; 6mm – 10mm, multifocal PanIN1–3, 5 well differentiated PNET; 2–15mm	Wild type	0	
4	Female / 58, BRCA2 mutant, CAPS3	MRI; 2 communicating cysts (tail -8mm)	2 communicating cysts (body, tail, 2–8mm), Dilated MPD; body, tail Dx; BD-IPMN	Distal panc / 2009	Pancreatic cyst	PanIN3	Multifocal PanIN1–3, IPMN low grade; 6mm	Wild type	0	
5	Female / 75, 1 FDR, 2 SDR, CAPS4	MRI; 2 communicating cysts (body 3–5mm) Dx: BD-IPMN	2 cysts (head, 3–5mm), Dilated MPD; echogenic, body, 3.7mm Dx; BD-IPMN, MPD stricture	Whipple / 2009	MPD stricture	PanIN3	Multifocal PanINs; PanIN1–3, PanIN3 involving MPD adjacent to MPD stricture	Mutant	6	5
6	Female / 46,	MRI; Cystic dilation of	3 communicating cysts (body, tail, 4–7mm), Dilated MPD; body, tail, 5mm	Distal panc / 2007	Pancreatic cyst	IPMN;	IPMN high grade; 10mm, IPMN low	Mutant	3	6
										12569 C>A, L188M

Gender / Age, Risk	Preoperative imaging			Surgery	Final pathologic diagnosis		TP53 in pancreatic juice		
	Contrast MRI or CT	EUS	Procedure / Year		Highest grade	Additional information	Status	Mut score	Mut Exon
PJS, CAPS2	branch ducts	Dx; BD-IPMN				grade; 5mm, 6 PanINs; PanIN1-2			
7 Male / 74, Control, CAPS3	CT; Cystic mass (head 53mm)	Cystic mass (head, 50mm) polypoid echogenic thickened wall (21mm) + septae Sub cm cysts (body), Dx; IPMN	Whipple / 2009	Pancreatic cyst	IPMN;	IPMN high grade; 50mm, PanIN2	Wild type	0	
8 Male / 49, Control, CAPS4	CT; Pancreatic cyst (uncinate 17mm) Dx: BD-IPMN	Pancreatic cyst (uncinate, 15mm) micro macrocystic. Dx: BD-IPMN	Whipple / 2010	Pancreatic cyst	IPMN;	MPD-IPMN high grade; 15mm	Mutant	3	6 12671 C>A, P222T
9 Male / 57, Control, CAPS3	CT; Cystic mass (body 17mm)	Complex septated cyst (body, 16mm) 8mm mural nodule, 6mm cyst (head), Dilated MPD; body, 3.8mm Dx: BD-IPMN	Distal panc / 2009	Pancreatic cyst	PanIN2	Focal PanIN2, Serous cystadenoma	Wild type	0	
10 Male / 63, 2 FDR, CAPS3	MRI: No evidence of pancreatic mass	Hypoechoic mass (tail, 12mm), Dx: pancreatic mass	Distal panc / 2010	Pancreatic mass	PanIN2	Multifocal PanIN; PanIN1-2, lobulocentric atrophy	Wild type	0	
11 Female / 57, Control, CAPS3	CT; Cystic mass (body 24mm)	2 cysts (head, body, 8-20mm) with thick septa, Dx: BD-IPMN or MCN	Distal panc. / 2007	Pancreatic cyst	IPMN;	BD-IPMN int-grade; 16mm, PanIN1	Wild type	0	
12 Male / 65 Control, CAPS3	MRI; Multiple cysts (largest head, 27mm) Dx: BD-IPMN	12 cysts (head, body, tail, largest 27mm, communicating) Dilated MPD Dx: multiple BD-IPMN	Whipple / 2008	Pancreatic cyst	IPMN; PanIN-2	Multiple BD-IPMN int-grade; largest size 30mm, PanIN1-2	Wild type	0	
13 Female / 66 Control, CAPS3	MRI; Multiple communicating cysts (largest 20mm) Dx: BD-IPMN	8 communicating cysts (head, body, -31mm) with multiple septa,	Whipple / 2009	Pancreatic cyst	IPMN;	BD-IPMN int-grade; 60mm	Mutant	2	8 13741 T>C, C275R

Gender / Age, Risk	Preoperative imaging		Surgery		Final pathologic diagnosis		TP53 in pancreatic juice	
	CAPS	Contrast MRI or CT	EUS	Procedure / Year	Indication for surgery	Highest grade	Status	Mut score
			Dx: multiple BD-IPMN					
14	Female / 66 Control, CAPS3	CT; Dilated CBD	CBD dilation (13mm) with polypoid, hypoechoic tissue, Mildly dilated MPD	Whipple / 2009	CBD dilation	IPMN; PanIN2	BD-IPMN int-grade; 6mm, Multifocal PanIN2	Wild type 0
15	Female / 62 Control, CAPS4	CT; 4 Cysts (head, body, tail, largest body, 10mm), Dx: IPMN	6 cysts (body, tail) largest (body) 13mm, irregular, septated. Dx: IPMNs	Distal panc / 2003	Pancreatic cyst	IPMN; PanIN2	MPD-IPMN int-grade; 15mm, Multifocal PanIN1-2	Wild type 0
16	Male / 65, 2 FDR CAPS4	MRI; Multiple cysts (largest body, 28mm) Dx: IPMN	Multiple cysts throughout pancreas (largest 28mm body), Dx: IPMNs	Distal panc / 2010	Pancreatic cyst	IPMN; PanIN2	BD-IPMN int-grade; 15mm, Multifocal PanIN2	Wild type 0
17	Male / 65, 1 FDR, 2 SDR, CAPS4	MRI; 2 communicating cysts, head 14-17mm, Dx: IPMN	2 cysts (head 19mm septated with 5mm mural nodule, head 10mm) Dx: IPMNs	Whipple / 2008	Pancreatic cyst	IPMN;	BD-IPMN int-grade; 15mm	Wild type 0
18	Male / 51, 1 FDR, 2 SDR, CAPS 2	MRI; Solid pancreatic mass (tail, 5mm)	Chronic pancreatitis, no pancreatic mass	Distal panc / 2006	Pancreatic mass	PanIN2	, Multifocal PanIN2, PNET 5mm	Wild type 0
19	Male / 77, 1 FDR, 2 SDR, CAPS 2	CT;	Cystic dilation of MPD (head, 11mm) Dx: IPMN	Whipple / 2004	Pancreatic cyst	PanIN2	Multifocal PanIN1-2	Wild type 0
20	Male / 60, 1 FDR, 2 SDR, CAPS 4	MRI; Pancreatic cyst (head, 4mm) Dx: IPMN	Periapillary hypoechoic mass 13mm Dx: PNET	Whipple / 2010	Periapillary mass	PanIN2	, Multifocal PanIN2, 2 islet cell tumors; 15mm, 12mm	Wild type 0
21	Female / 78, 1 FDR, 2 SDR, CAPS 4	MRI; 3 communicating cysts (head, body, tail 4-10mm)	Multiple cysts (body, tail, largest 6mm) Dx: IPMNs	Distal panc / 2010	Pancreatic cyst	PanIN2	Multifocal PanIN-2 associated lobulocentric atrophy, 4 incipient IPMNs low-grade; 4mm	Wild type 0

Gender / Age, Risk	Preoperative imaging			Surgery	Final pathologic diagnosis			TP53 in pancreatic juice		
	CAPS	Contrast MRI or CT	EUS	Procedure / Year	Indication for surgery	Highest grade	Additional information	Status	Mut score	Exon
22	Female / 61, 2 FDR, CAPS4	MRI; Communicating cyst (tail, 4mm) Dx; IPMN	Hypoechoic solid mass (tail, 8mm) Dx; PNET	Distal pan / 2010	Pancreatic mass	PanIN2	Multifocal PanIN-2 associated lobulocentric atrophy	Wild type	0	
23	Male / 48 Control, CAPS2	CT; Pancreatic cyst 15mm (uncinate) Dx: BD-IPMN	2 cysts (head) largest 18mm increasing size. MPD dilated, echogenic, Dx; BD-IPMN	Whipple / 2004	Pancreatic cyst	PanIN2	Multifocal PanIN1-2, 2 BD-IPMN low grade; 10mm 15mm	Wild type	0	
24	Male / 48, 1 FDR, 2 SDR, CAPS 2	MRI, CT; No pancreatic mass	Cystic dilation of MPD (head, 14mm) Dx; IPMN	Whipple / 2004	Pancreatic cyst	IPMN; low-grade PanIN1B	BD-IPMN low-grade; 12mm, Focal PanIN1B	Wild type	0	
25	Male / 65, 1 FDR, 2 SDR, CAPS 4	MRI; 2 communicating cyst (head 24mm, tail 18mm) Dx: BD-IPMN	2 cysts (head 28mm septated, body 9mm) Dx; BD-IPMN's	Distal pan / 2009	Pancreatic cyst	IPMN; low-grade	BD-IPMN low-grade; 20mm	Wild type	0	
26	Female / 78 Control, CAPS3	MRI; Communicating cyst (head, 13mm) Dx: BD-IPMN	A communicating cyst (head, 8mm) Dx; BD-IPMN	Whipple / 2008	Pancreatic cyst	IPMN;	BD-IPMN low grade; 5mm	Wild type	0	
27	Male / 56, Control, CAPS4	CT; Dilated CBD (17mm)	CBD dilation, no mass Dx; Dilated CBD	Whipple / 2010	CBD dilation	PanIN1B	Pancreas with duct ectasia and focal PanIN1B	Wild type	0	
28	Male / 45 Control, CAPS4	MRI; Multifocal mass (tail, 57mm) Dx; serous cystadenoma	A cystic mass (tail, 50mm) consisted of soft tissue and tiny cystic compartments Dx; serous cystadenoma	Distal pan / 2009	Pancreatic cyst	PanIN1A	Focal PanIN1A, Serous cystadenoma; 50mm	Wild type	0	

IPMN, intra-ductal papillary mucinous tumor; PanIN, pancreatic intraepithelial neoplasia; CAPS, Cancer of the Pancreas Screening; FDR, first degree relative; SDR, second degree relative; BD, branch duct; MPD, main pancreatic duct; CT, computed tomography; MRI, magnetic resonance imaging; EUS, endoscopic ultrasonography; CBD, common bile duct; int, intermediate; panc, pancreatotomy; Dx, Diagnosis; Mut, mutation.