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Translational Cancer Mechanisms and Therapy

Clinical Cancer Research

Pancreatic Juice Mutation Concentrations Can Help Predict the Grade of Dysplasia in Patients Undergoing Pancreatic Surveillance

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Abstract

Purpose: The measurement of mutations in pancreatic juice samples collected from the duodenum during endoscopic ultrasound (EUS) may improve the diagnostic evaluation of patients undergoing pancreatic surveillance. Our aim was to evaluate the accuracy of using pancreatic juice mutation concentrations to predict the presence and histologic grade of neoplasia in the pancreas.

Experimental Design: Digital next-generation sequencing (NGS) of pancreatic juice DNA using a targeted 12-gene panel was performed on 67 patients undergoing pancreatic evaluation during EUS, including patients with pancreatic ductal adenocarcinoma, patients who subsequently underwent pancreatic resection for precursor lesions, patients undergoing surveillance for their familial/inherited susceptibility to pancreatic cancer, and normal pancreas disease controls.

Results: Patients with pancreatic cancer or high-grade dysplasia as their highest grade lesion had significantly higher pancreatic juice mutation concentrations than all other subjects (mean/SD digital NGS score; 46.6 ± 69.7 vs. 6.2 ± 11.6, $P = 0.02$). Pancreatic juice mutation concentrations distinguished patients with pancreatic cancer or high-grade dysplasia in their resection specimen from all other subjects with 72.2% sensitivity and 89.4% specificity [area under the curve (AUC) = 0.872]. Mutant *TP53*/*SMAD4* concentrations could distinguish patients with pancreatic cancer or high-grade dysplasia in their resection specimen from all other subjects with 61.1% sensitivity and 95.7% specificity (AUC = 0.819). Among 31 high-risk individuals under surveillance, 2 of the 3 individuals with most abnormal pancreatic juice mutation profiles also had the most abnormalities on pancreatic imaging.

Conclusions: Pancreatic juice mutation analysis using digital NGS has potential diagnostic utility in the evaluation of patients undergoing pancreatic surveillance. *Clin Cancer Res; 24(12); 2963–74.* *©2018 AACR.*

See related commentary by Lipner and Yeh, p. 2713

Introduction

Pancreatic cancer is expected to be the second leading cause of cancer-related deaths by the year 2030 in the United States (1). Most patients with pancreatic ductal adenocarcinoma (PDAC) present with advanced-stage cancers and have rapidly progressive disease (2).

Pancreatic screening targets high-risk individuals, and a major determinant of the risk/benefit of pancreatic screening is optimal pancreatic cancer risk assessment. Pancreatic cancer risk assessment uses family history of pancreatic cancer, patient age, and germline mutation status to assess risk. The identification of additional risk factors, including biomarkers of risk (3), would permit better selection of candidates for pancreatic screening. To date, studies evaluating pancreatic screening have found that endoscopic ultrasound (EUS) and magnetic resonance imaging/magnetic resonance cholangiopancreatography (MRI/MRCP) frequently identify pancreatic cysts, although these are usually of low-malignancy potential (4–12). Initial studies have also found that pancreatic screening may result in downstaging of screen-detected pancreatic cancers (13, 14), but detecting pancreatic cancers at low stage may not prevent pancreatic cancer–related death and the emergence of interval cancers despite adequate surveillance, highlighting the limitations of existing pancreatic screening tests.

The frequent detection of small pancreatic cysts consistent with intraductal papillary mucinous neoplasm(s) (IPMN) in patients undergoing screening may give the impression that IPMN is the major precursor lesion in these patients. However, high-risk individuals who undergo pancreatic resection usually have more pancreatic intraepithelial neoplasia (PanIN) than IPMN in their resected pancreata, and most of these PanINs are not detected by pancreatic imaging tests (15). Pancreatic imaging also does not reliably grade IPMNs and can miss small (subcentimeter) cancers (5, 6, 12). Annual surveillance is recommended for most

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Translational Relevance

Pancreatic imaging cannot reliably grade dysplasia in the pancreas, which makes pancreas surveillance challenging. Mutational analysis of pancreatic juice has the potential to provide evidence of the presence or absence of dysplasia or cancer not always evident by imaging. In this study, we evaluated the performance of a digital next-generation sequencing assay designed to detect low-abundance mutations in pancreatic juice collected from the duodenum of patients undergoing endoscopic ultrasound evaluation of the pancreas for pancreatic surveillance and other indications. We find that overall pancreatic juice mutation concentrations and concentrations of mutations in *TP53*/*SMAD4* are accurate predictors of neoplastic grade (i.e., the presence or absence of high-grade dysplasia and/or invasive pancreatic cancer). Pancreatic juice analysis is a promising diagnostic test for the evaluation of dysplasia in patients undergoing pancreatic surveillance.

individuals because longer screening intervals would likely lead to more interval cancers (16, 17). Since a major goal of pancreatic screening is to detect and resect PanIN and IPMN with high-grade dysplasia (17, 18), tests that could better reveal the extent of neoplasia in the pancreas would enable more optimal decisions about surveillance intervals and surgical decision-making. One test that could help with pancreatic surveillance is a pancreatic juice mutation test. Analysis of secretin-stimulated pancreatic juice collected endoscopically from the duodenum of patients enrolled in the Cancer of the Pancreas Screening (CAPS) trials revealed that these samples contain mutations that reflect the burden of neoplasia in the pancreas (19–23). In a prior study, we reported our initial experience with using a next-generation sequencing (NGS) method, termed "digital NGS," to detect low-abundance mutations (19). Interpreting pancreatic juice mutation profiles can be guided by what is known about the timing of mutations in pancreatic cancer precursor lesions. *KRAS* mutations emerge in PanIN-1 (~90% of PanIN-1 lesions harbor *KRAS* mutations; ref. 24); similarly, *GNAS* and *KRAS* mutations generally emerge in IPMNs with low-grade dysplasia (25, 26); *TP53* mutations generally emerge with high-grade dysplasia (i.e., PanIN-3 or IPMN with high-grade dysplasia; ref. 21), and biallelic inactivation of *SMAD4* occurs in lesions with high-grade dysplasia and may promote the transition to invasive cancer (27). Approximately 90% of pancreatic cancers harbor *KRAS* mutations, approximately 70% harbor *TP53* mutations, and approximately 20% harbor *SMAD4* mutations (another approximately 30% have homozygous deletion of *SMAD4*; ref. 28). Other genes including *CDKN2A*, *RNF43*, *ARID1A*, and *TGFBR2*, which harbor intragenic mutations in approximately 5% to 20% of PDACs (21, 29), are thought to have variable timing of mutation in pancreatic precursor lesions.

In this study, we employed an improved digital NGS assay (deeper sampling and more genes) and evaluated its potential utility as a test for high-risk individuals under surveillance, comparing their mutation profiles to those from patients with pancreatic cancer, normal pancreas disease controls, and patients who underwent pancreatic resection for suspected precancerous lesions.

Materials and Methods

Patients and specimens

Pancreatic fluid samples and clinical information were obtained from participants enrolled in the CAPS studies (NCT00714701 and NCT02000089; ref. 12). For this study, we evaluated 67 subjects enrolled at Johns Hopkins Hospital who had sufficient pancreatic fluid samples for analysis (Table 1). None of these cases were included in our prior study evaluating pancreatic juice digital NGS (19). As the most important goal of pancreatic surveillance is to detect evidence of pancreatic cancer or high-grade dysplasia, patients were grouped into those with pancreatic cancer or high-grade dysplasia (PanIN-3 or in IPMN) versus all others (disease controls, those under surveillance, and those with low-grade dysplasia). Patients included (i) those with pancreatic cancer ($n = 14$); (ii) those who underwent surgical resection that had precursor lesions with low-grade or high-grade dysplasia ($n = 13$); (iii) those under surveillance for familial/inherited risk ("surveillance group"; $n = 31$); and (iv) disease controls with normal pancreata ($n = 9$; ref. 17). Patients with a familial/inherited risk considered appropriate for surveillance were (i) those with a family history of pancreatic cancer in at least 2 affected blood relatives with pancreatic cancer related by first degree), (ii) germline mutation carriers (*BRCA2*, *PALB2*, *ATM*, and *BRCA1*) with a family history of pancreatic cancer, or (iii) those with inherited cancer syndromes irrespective of their pancreatic cancer family history [*CDKN2A* (familial atypical melanoma mole syndrome) and *PRSS1* (hereditary pancreatitis) mutation carriers, and Peutz–Jeghers syndrome (PJS)]. Pancreatic juice was obtained from high-risk individuals in the surveillance group at least 2 years prior to their last follow-up. Low-grade IPMN, PanIN-1, intermediate-grade IPMN, and PanIN-2 lesions were classified as low-grade precursors per consensus recommendations (30). As the whole pancreas is not evaluated in patients who undergo partial pancreatectomy, the extent and grading of dysplasia are incomplete (30). Further description of the study population is provided in Table 1.

Pancreatic juice samples were collected from all participants after intravenous human synthetic secretin infusion (0.2 μg/kg over 1 minute). No adverse events were associated with secretin administration. Juice was collected from the duodenal lumen through the endoscopic channel over 5 minutes (typically 5–10 mL; ref. 12). We recently evaluated the use of an endoscopic cap to collect pancreatic juice, but the endoscopic cap was not used in this study (31). Juice samples were stored at −80°C before use. DNA was extracted using the DNeasy blood and tissue kit (QIAGEN). All study elements were approved by the Johns Hopkins institutional review board, and written informed consent was obtained from all patients.

Digital NGS

All digital NGS assays were performed blinded to patient information. An Ion AmpliSeq custom panel was employed to multiplex PCR (94 amplicons in two primer pools; see Supplementary Table S1) and sequence 12 genes (*KRAS*, *GNAS*, *TP53*, *SMAD4*, *CDKN2A*, *RNF43*, *TGFBR2*, *BRAF*, *PIK3CA*, *ARID1A*, *FBXW7*, and *VHL*; *VHL* is mutated in serous cystadenomas but not expected to be mutated in pancreatic ductal neoplasms). Homozygous deletions involving *SMAD4* or other genes are not detectable in secondary fluids with NGS assays. (Custom assays could potentially detect structural deletions in secondary fluids if

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Table 1. Characteristics of cases included in this study

Case #	M/F	Age	CAPS #	Risk group	Study group	Lesion	# of lesions	Largest lesion (mm)	EUS	Diagnosis
#104	F	84	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Suspicion of dilated CBD
#108	M	46	CAPS5	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Suspicion of a pancreatic lesion by CT
#109	F	54	CAPS5	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Abdominal pain
#122	M	59	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Weight loss
#123	F	47	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Duodenal diverticulum, history of acute pancreatitis
#131	F	56	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	A lesion adjacent to celiac artery by CT
#134	M	76	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Suspicion of a pancreatic lesion by CT
#135	F	69	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	Suspicion of a cystic lesion by EUS 3 years ago
#137	F	38	CAPS4	Control	Disease control	Normal pancreas	na	na	Normal pancreas	CBD stone
#089	F	74	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#091	F	79	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#093	M	71	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#095	M	78	CAPS4	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#099	M	72	CAPS4	High risk—BRCA2	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#121	M	67	CAPS4	High risk—BRCA2	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#124	F	70	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#105	M	70	CAPS4	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#127	M	70	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#128	M	73	CAPS4	High risk—BRCA1	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#129	F	70	CAPS4	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#130	F	69	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#132	F	67	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#133	F	65	CAPS5	Familial	Surveillance	No mass, no cyst	na	na	Normal pancreas	CAPS surveillance
#090	M	77	CAPS5	High risk—p16	Surveillance	Cyst	1	7	Cyst in tail	IPMN suspected by EUS
#092	M	74	CAPS5	Familial	Surveillance	Cysts	4	18	Cysts in body and head	IPMN suspected by EUS
#096	M	71	CAPS4	Familial	Surveillance	Cysts	2	5	Cysts in body and tail	IPMN suspected by EUS
#100	M	72	CAPS4	Familial	Surveillance	Cysts	3	7	Cysts in body	IPMN suspected by EUS
#110	M	75	CAPS5	Familial	Surveillance	Cysts	6	14	Cysts in head, body and tail	IPMN suspected by EUS
#111	M	70	CAPS5	Familial	Surveillance	Cysts	2	15	Cysts in body and head	IPMN suspected by EUS
#112	M	72	CAPS4	Familial	Surveillance	Cysts	2	11	Cysts in head and body	IPMN suspected by EUS
#113	F	58	CAPS4	Familial	Surveillance	Cysts	3	11	Cysts in body	IPMN suspected by EUS
#114	M	63	CAPS4	Familial	Surveillance	Cysts	3	16	Cysts in tail, head and body	IPMN suspected by EUS
#115	M	62	CAPS5	High risk—PJS	Surveillance	Cysts	3	17	Cysts in head, body and tail	IPMN suspected by EUS
#116	F	38	CAPS5	High risk—PJS	Surveillance	Cysts	4	17	Cysts in head, tail and body	IPMN suspected by EUS
#117	F	69	CAPS5	Familial	Surveillance	Cysts	5	11	Cysts in head, tail, body, 5 mm hypoechoic lesion in tail	IPMN suspected by EUS, follow-up a hypoechoic lesion
#118	F	53	CAPS5	High risk—BRCA2	Surveillance	Cyst	1	12	Cyst in body	IPMN suspected by EUS
#119	F	68	CAPS5	Familial	Surveillance	Cysts	7	15	Cysts in head and tail	IPMN suspected by EUS
#120	F	54	CAPS5	Familial	Surveillance	Cyst	1	12	Cyst in tail	IPMN suspected by EUS
#126	F	72	CAPS5	Familial	Surveillance	Cyst—5 mm			Cyst in uncinate	CAPS surveillance
#136	F	57	CAPS5	High risk—PALB2	Surveillance	Cysts	13	12	Cysts in head, tail and body	IPMN suspected by EUS
#077	M	57	CAPS5	Incidental	Precursor—LG	Mass	1	34	Normal pancreas, no evidence of mass	Pathology: chronic pancreatitis, PanIN-2

(Continued on the following page)

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Table 1. Characteristics of cases included in this study (Cont'd)

Case #	M/F	Age	CAPS #	Risk group	Study group	Lesion	# of lesions	Largest lesion (mm)	EUS	Diagnosis
#072	F	58	CAPS4	High risk—PRSS1	Precursor—LG	Cyst	1	12	Cyst in body, chronic pancreatitis, 6-mm dilated MPD	Pathology: IPMN IGD, chronic pancreatitis, PanIN-2
#075	M	64	CAPS4	High risk—BRCA2	Precursor—LG	Solid lesion	1	14	Hypoechoic lesion in tail	Pathology: Chronic pancreatitis, PanIN-2
#076	M	69	CAPS5	Familial	Precursor—LG	Cysts	6	6	Cysts in body and tail, 7-mm dilated MPD	Pathology: IPMN IGD, PanIN-1B
#107	F	61	CAPS5	Incidental	Precursor—LG	MPD dilatation	1	10	Dilated MPD, 10 mm	Pathology: IPMN IGD, PanIN-2
#087	M	65	CAPS5	Incidental	Precursor—LG	Cyst	1	34	Cyst in body, 5-mm dilated MPD	Pathology: IPMN LGD
#071	M	65	CAPS4	Familial	Precursor—LG	Cysts	Many	28	Cysts in body, head and tail	Pathology: IPMN IGD, PanIN-2
#070[a]	M	62	CAPS4	Familial	Precursor—ERCP	Cysts	2	5	Cysts in head and body, 6-mm dilated MPD	Pathology: incipient IPMN, PanIN-2
#079	M	57	CAPS5	McCune–Albright syndrome	Precursor—McCune–Albright syndrome	Cysts	Many	17	Cysts in head, body and tail, 13-mm dilated MPD	McCune-Albright syndrome, Pathology: IPMN LGD
#073	F	66	CAPS4	Familial	Precursor—HG	Cysts	12	17	Cysts in body, head and tail	Pathology: multifocal PanIN with focal HGD (PanIN-3)
#074	F	65	CAPS4	High risk—PJS	Precursor—HG	Cysts	4	10	Cysts in head and body	Pathology: IPMN HGD, PanIN-2
#081	F	87	CAPS5	Incidental	Precursor—HG	Cyst	1	28	Cyst with solid component in head	Pathology: IPMN HGD
#098	M	77	CAPS4	Incidental	Precursor—HG	Cysts	2	21	Cysts in head and body	Pathology: IPMN HGD, PanIN-2
#078	F	75	CAPS5	PC	PC	Mass	1	40	Mass in head, 10-mm dilated MPD	Pathology: PDAC, stage IV, diagnosed by FNA
#080	F	78	CAPS5	PC	PC	Mass	1	36	Mass in head	Pathology: PDAC, ypStage IB
#082	M	62	CAPS5	PC	PC	Mass	1	32	Mass in head, 4-mm dilated MPD	Pathology: PDAC, stage IV, diagnosed by FNA
#083	F	72	CAPS5	PC	PC	Mass	1	37	Mass in head, 6-mm dilated MPD	Pathology: PDAC, stage IIB
#084	M	72	CAPS5	PC	PC	Cyst	1	36	Cyst with 12-mm solid component in head	Pathology: PDAC arising in an IPMN, stage IA, IPMN, PanIN
#085	M	73	CAPS5	PC	PC	Mass	1	49	Mass in head, 10-mm dilated MPD	Pathology: PDAC, stage IIB, PanIN-2
#086	M	65	CAPS5	PC	PC	Mass	1	27	Mass in body, 8-mm dilated MPD	Pathology: PDAC, ypStage IIB
#088	F	77	CAPS5	PC	PC	Mass	1	29	Mass in head, 6-mm dilated MPD	Pathology: PDAC, locally advanced, diagnosed by FNA
#094	M	53	CAPS5	PC	PC	Mass	1	30	Mass in body, 5-mm dilated MPD	Pathology: PDAC, locally advanced, diagnosed by FNA
#097	M	51	CAPS5	PC	PC	Mass	1	24	Mass in head, 6-mm dilated MPD	Pathology: PDAC, ypStage IIB
#101	M	85	CAPS4	PC	PC	Mass, cyst	2	35	Mass in tail, 4-mm cyst in body, 4-mm dilated MPD	Pathology: colloid carcinoma arising in an IPMN, stage IA, IPMN HGD
#102	M	68	CAPS4	PC	PC	Mass	1	30	Mass in head, 7-mm dilated MPD	Pathology: PDAC, stage IIB
#103	M	61	CAPS4	PC	PC	Cyst	1	31	Cyst with solid compornent in body, 7-mm dilated MPD	Pathology: PDAC arising in an IPMN, stage IB, IPMN HGD
#106	F	62	CAPS4	PC	PC	Mass	1	25	Mass in tail, 5-mm dilated MPD	Pathology: PDAC, stage IIB

Abbreviations: CBD, common bile duct; CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; F, female; FNA, fine-needle aspiration; HG, high grade; HGD, high-grade dysplasia; IGD, intermediate-grade dysplasia; LG, low grade; LGD, low-grade dysplasia; M, male; MPD, main pancreatic duct; na, not applicable; PC, pancreatic cancer; Precursor, surgical resection for precursors; ypStage, pathologic stage after neoadjuvant therapy.

[a]ERCP.

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the structural deletion has been characterized after sequencing the primary tumor). To increase the limit of detection of digital NGS, 192 individual aliquots of DNA (100 pg/well) were individually sequenced from each patient's juice sample (twice as many as in our prior study; ref. 19). Sequencing depth was also increased by using Ion Proton chips. The average read coverage in aliquots with gene mutations was 1,551 (interquartile range, 1,040–2,222). Each pancreatic juice library was subjected to approximately 30 million reads, approximately 300,000 reads per amplicon (192 NGS reactions × 94 amplicons × ~1,500 reads/amplicon/well). Libraries were generated using Ion AmpliSeq library kit 2.0, amplified by Ion OneTouch 2 and enriched by Ion OneTouch ES (all from Thermo Fisher Scientific). Libraries were prepared and loaded onto Ion PI chips and sequenced using an Ion Proton system (Thermo Fisher Scientific) following the manufacturer's protocols.

Digital NGS data analysis

Postsequencing data analyses, including alignment to the hg19 human reference genome and variant calling, were performed using NextGENe software (v2.4, SoftGenetics). Alignments and putative mutations were visually verified using Integrative Genomics Viewer [(IGV) v2.3, Broad Institute] and NextGENe Viewer.

NGS variants were considered true only when: (i) read coverage at the variant position was at least 300, (ii) balance ratio (the ratio of the forward vs. reverse reads with the variant) was 0.1 or greater and, (iii) the quality score of candidate mutation calls was ≥30. Then, digital NGS scores for each candidate mutation were counted for each sample; mutation score of 1 is given for each NGS aliquot with a mutation (with 192 NGS results per juice sample, the maximum score for any mutation was 192). Digital NGS scores increase exponentially with mutation concentration: As 100 pg/DNA is sampled for each NGS result, a juice sample with a mutation concentration of approximately 0.1% would be predicted to have a digital NGS score of approximately 5 to 7; for a sample with a mutation concentration of 1%, the score would be approximately 50 to 70. To address false-positive mutation calls arising from NGS errors, the number of mutation-positive wells required for a sample to be considered as a true mutation was set as four except for hot spot mutations. This cutoff was above background false-positive levels generated in negative control samples including fibroblast, CAF19 DNA, and other samples reported previously (19). For *TP53* hot spot mutations (R175H, G245D, G245S, R248Q, R248W, R249S, R273C, R273H, and R282W), three positive wells were required to be interpreted as having a mutation. Although prevalent, some hot spots (*KRAS* G12D, and R201C and R201H in *GNAS*) are subject to rare cytosine deamination events (32); based on our prior experience, we set two positive wells for *KRAS* G12D and R201C and R201H in *GNAS* and one positive well for *KRAS* G12V G12R mutations as having a mutation (19, 31). For other *KRAS* hot spot mutations, we set the threshold as two positive wells as positive.

Recurrent non–hot spot mutations occurring in more than 3 patient samples were considered likely sequencing error related; synonymous somatic mutations were not considered deleterious and were not tabulated. The potential pathogenicity of somatic mutations was evaluated using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and COSMIC (http://grch37-cancer.sanger.ac.uk/cosmic), with FATHMM scores of 0.9 or higher as criteria for pathogenicity. Variants judged as benign were not tabulated. Variants of uncertain significance (VUS), listed in Supplementary Table S2, were excluded from further analysis.

Statistical analysis

Qualitative variables were compared by χ^2 tests, and quantitative variables were compared by Mann–Whitney *U* test. The main goal was to compare patients with pancreatic cancer or high-grade dysplasia to those without evidence of pancreatic cancer or high-grade dysplasia. Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic accuracy of digital NGS scores to distinguish between these diagnostic groups, and the area under the curve (AUC) was computed by the trapezoidal method. Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software). $P < 0.05$ was considered statistically significant.

Results

Pancreatic juice mutation results are shown in Fig. 1 and Table 2. A total of 155 unique somatic mutations were detected. Numerous VUS were also detected (listed in Supplementary Table S2). Pancreatic juice mutations were detected in all 14 cases with pancreatic cancer, 12 of 13 (92.3%) cases with suspected precancerous lesions, 23 of 31 high-risk individuals (74.2%) undergoing pancreatic surveillance, and 4 of 9 disease controls with a normal pancreas. One disease control with pancreatic juice mutations had a history of idiopathic acute pancreatitis. Two cases [1 with McCune–Albright syndrome and 1 whose pancreatic juice was collected from the main pancreatic duct during endoscopic retrograde cholangiopancreatography (ERCP)] that had additional reasons for having very high pancreatic juice mutation concentrations were included for illustration but not in the formal evaluation of the diagnostic accuracy of the juice test (further described below).

Forty-eight of 67 patients had *KRAS* and/or *GNAS* mutations, including 2 of 9 normal pancreas controls, 21 of 31 high-risk individuals under surveillance, 11 of 13 individuals who had undergone pancreatic resection for precursor lesions, and 12 of 14 patients with pancreatic cancer. *GNAS* mutations were detected in 7 of the 10 patients who had IPMNs at resection. Overall, normal pancreas controls were less likely to have *KRAS* and/or *GNAS* mutations than all others (3 of 9 vs. 45 of 57, $P = 0.01$, Fisher exact test). High-risk individuals with pancreatic cysts tended to be more likely to have mutations detected in their juice samples than those without cysts ($P = 0.1$, Fisher exact test). The median total mutation score among high-risk individuals with cysts was higher (6 ± 15 vs. 1.5 ± 2.4) than for high-risk individuals without cysts ($P < 0.05$, Mann–Whitney *U* test).

Most (26 of 31) subjects who had a mutation in a gene other than *KRAS* or *GNAS* also had one or more *KRAS* and/or *GNAS* mutations. Patients with pancreatic cancer or with high-grade dysplasia in their resection specimen were more likely than disease controls to have a mutation other than *KRAS/GNAS* (15 of 18 cases vs. 3 of 9 controls, $P = 0.026$, Fisher exact test). Four of 9 patients who underwent pancreatic resection without high-grade dysplasia, 6 of 17 high-risk individuals with cysts, and 3 of 14 high-risk individuals without cysts also had mutations in genes other than *KRAS/GNAS*.

Patients with pancreatic cancer or high-grade dysplasia were more likely than all other subjects to have higher concentrations of mutations other than *KRAS/GNAS* ($P < 0.0001$). The most

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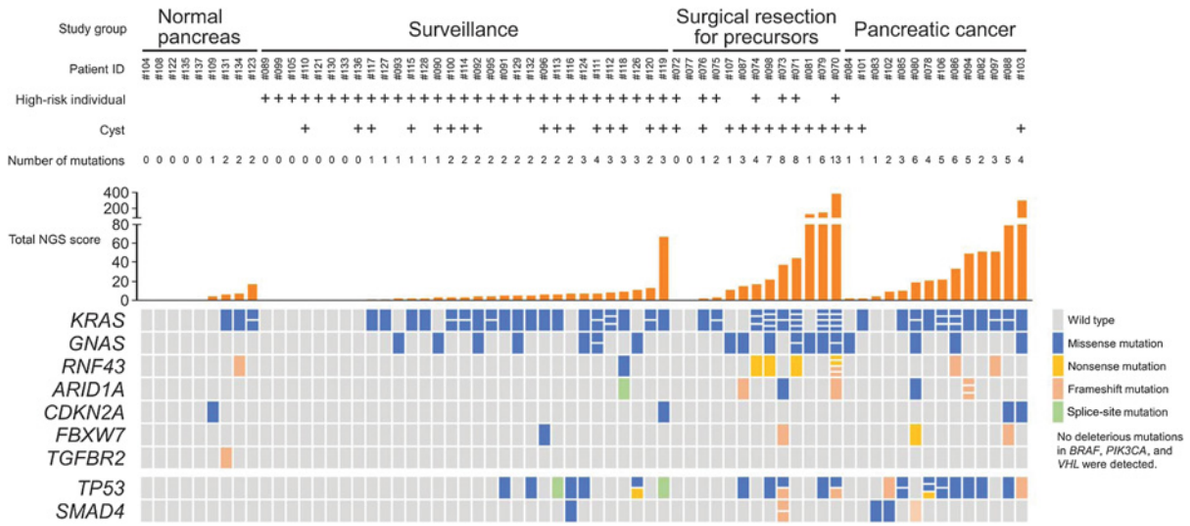

Figure 1.

Summary of pancreatic juice mutation profiles. The mutational status of each gene is listed, along with information about study group, pancreatic cyst status, the number of mutations, and the total digital NGS score for each sample. One tile on the bottom half of the figure represents the mutation results for that gene in that sample.

commonly mutated genes after *KRAS* and *GNAS* were, in descending order of prevalence, *TP53*, *RNF43*, *SMAD4*, *ARID1A*, *CDKN2A*, *FWXB7*, and *TGFBR2*. No deleterious mutations were detected in *VHL*, *BRAF*, or *PIK3CA*. Consistent with a recent study on concurrent mutations in cancer, there was a significant association between having an *ARID1A* and an *RNF43* mutation ($P = 0.044$, Fisher exact test; ref. 33).

SMAD4 mutations were the most specific of the mutations for having pancreatic cancer or an advanced precursor lesion, confirming the results of our previous study (19). Only 5 patients had a *SMAD4* mutation—3 with PDAC, 1 with high-grade dysplasia, and 1 high-risk individual with PJS with five cysts undergoing close surveillance. Twenty-one patients had *TP53* mutations, including 9 patients with PDAC; 2 patients with high-grade dysplasia; the patient with McCune–Albright syndrome who had extensive precancerous disease; 1 patient with multifocal PanIN-2 in his or her resection specimen; 1 patient with a 3.4-cm diameter main-duct IPMN; and 7 high-risk individuals under surveillance, including 1 with PJS with five cysts and 1 with high pancreatic juice *KRAS* mutation concentrations (digital NGS score of 59). No *TP53* or *SMAD4* mutations were found in disease controls. Consistent with the molecular progression model of pancreatic neoplasia, where mutations in *TP53* and/or *SMAD4* emerge with higher grades of dysplasia, mutations involving either *TP53* and/or *SMAD4* were found in 13 of the 18 cases that had pancreatic ductal adenocarcinoma or high-grade dysplasia. In contrast, among cases where the grade of neoplasia in the pancreas was known or predicted to be low or absent (in disease controls with normal pancreata), none of 9 controls and 1 of the 7 cases that had low- or intermediate-grade dysplasia as their highest grade of neoplasia in their resection had *TP53* and/or *SMAD4* mutations ($P = 0.00001$).

The combined *SMAD4/TP53* mutation score of $\geq$5 was detected in 11 of the 18 pancreatic cancer/high-grade dysplasia cases versus 2 for all 47 others (sensitivity 61.1%, specificity 95.7%, AUC of 0.819 by ROC curve analysis; Fig. 2). The only nonpancreatic cancer/high-grade dysplasia cases that had this high of a mutation score were 2 subjects in the high-risk surveillance group with abnormal pancreatic imaging findings: the PJS case with five pancreatic cysts (case #116) and a high-risk individual with being cysts, with the largest being 17 mm (case #126).

Patients with pancreatic cancer or high-grade dysplasia as their highest grade lesion had significantly higher digital NGS scores than all other subjects (46.6 ± 69.7 vs. 6.2 ± 11.6, $P = 0.02$). Using an overall digital NGS score of 12 or higher as a cutoff (chosen to optimize diagnostic specificity), 13 of 18 patients who had pancreatic cancer or high-grade dysplasia as their highest grade lesion were positive versus 5 of the 47 remaining subjects (sensitivity, 72.2%, specificity, 89.4%). These 5 patients with digital NGS scores $\geq$12 included 1 patient who underwent pancreatic resection for multiple pancreatic cysts including an IPMN with intermediate-grade dysplasia (case #71), a high-risk individual under surveillance with cysts who also had a positive *TP53/SMAD4* mutation score (case #87), the disease control patient with a past history of acute pancreatitis who had only *KRAS* mutations in her juice sample (case #123), and the high-risk individual under surveillance who had a high *KRAS* mutation score (case #119). Lowering the digital NGS cutoff score to 9 increased diagnostic sensitivity to 77.7% but reduced diagnostic specificity to 83%. By ROC curve analysis, the overall digital NGS score distinguished patients with pancreatic cancer or high-grade dysplasia from all others, with an AUC of 0.872 (Fig. 2). Similar results were obtained if *KRAS/GNAS* mutations were excluded from the digital NGS mutation score; a digital NGS score without *KRAS/GNAS* mutations of 7 or higher yielded the same result (sensitivity, 72.2%, specificity, 89.4%; Fig. 2). Considering either a total digital NGS score of $\geq$12 or a *TP53/SMAD4* score of $\geq$5 increased diagnostic sensitivity to 83.3% but reduced diagnostic specificity to 87.2%. The number of unique mutations in pancreatic

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Table 2. Somatic mutations detected in pancreatic juice by digital NGS

					KRAS		*GNAS*		*RNF43*		*TP53*		*SMAD4*				
Case#	**M/F**	**Age**	**Risk**	**Study group**		**dNGS#**		**dNGS#**		**dNGS#**		**dNGS#**		**dNGS#**	**Other[*] dNGS#**	**Total dNGS#**	**# of unique mutations**
104	F	84	Control	Normal												**0**	
108	M	46	Control	Normal												**0**	
109	F	54	Control	Normal											4	**4**	1
122	M	59	Control	Normal												**0**	
123	F	47	Control	Normal	p.G12D, G13D	5,12										**17**	2
131	F	56	Control	Normal	p.G12V	2									4	**6**	3
134	M	76	Control	Normal	p.G12R	1			p.L163fs	6						**7**	2
135	F	69	Control	Normal												**0**	
137	F	38	Control	Normal												**0**	
89	F	74	Familial	Surveillance												**0**	
91	F	79	Familial	Surveillance	p.Q61R	2					p.R273H	3				**5**	2
93	M	71	Familial	Surveillance			p.R201C	2								**2**	1
95	M	78	Familial	Surveillance	p.G12V/S	1,3										**4**	2
99	M	72	HR—BRCA2	Surveillance												**0**	
121	M	67	HR—BRCA2	Surveillance												**0**	
124	F	70	Familial	Surveillance	p.G12V	1	p.R201C	2			p.H214R	4				**7**	3
105	M	70	Familial	Surveillance												**0**	
127	M	70	Familial	Surveillance	p.G12R	1										**1**	1
128	M	73	HR—BRCA1	Surveillance	p.Q61R	2										**2**	1
129	F	70	Familial	Surveillance	p.G13D	3	p.R201H	2								**5**	2
130	F	69	Familial	Surveillance												**0**	
132	F	67	Familial	Surveillance	p.G12V	1					p.H179R	4				**5**	2
133	F	65	Familial	Surveillance												**0**	
90	M	77	HR—p16	Surveillance			p.R201H	3								**3**	1
92	M	74	Familial	Surveillance	p.G12D	2	p.R201C	2								**4**	2
96	M	71	Familial	Surveillance	p.G12V	2									4	**6**	2
100	M	72	Familial	Surveillance	p.G12D/V	2,1										**3**	2
110	M	75	Familial	Surveillance												**0**	
111	M	70	Familial	Surveillance	p.G12V/R	2,1	p.R201C/H	2,2								**7**	4
112	M	72	Familial	Surveillance	p.G12D/S, Q61H[a]	2,3,3										**8**	3
113	F	58	Familial	Surveillance	p.G12D	2					c.375+2A>AG	4				**6**	2
114	M	63	Familial	Surveillance	p.G12V,Q61R	1,2										**3**	2
115	M	62	HR—PJS	Surveillance	p.G12V	2										**2**	1
116	F	38	HR—PJS	Surveillance							p.R273H	3	p.C499R	4		**7**	2
117	F	69	Familial	Surveillance	p.G12R	1										**1**	1
118	F	53	HR—BRCA2	Surveillance	p.G12R	1			p.S474P	4					4	**9**	3
119	F	68	Familial	Surveillance	p.G12D	59					c.559+2A>AG	4			4	**67**	3
120	F	54	Familial	Surveillance	p.G12A,Q61H[a]	4,9										**13**	2
126	F	72	Familial	Surveillance			p.R201H	2			p.R248W, R306X	5,4				**11**	3
136	F	57	HR—PALB2	Surveillance												**0**	
77	M	57	Incidental	Precursor—LG												**0**	
75	M	64	HR—BRCA2	Precursor—LG	p.G12V, Q61R	1,2										**3**	2
72	F	58	HR—PRSS1	Precursor—LG												**0**	
76	M	69	Familial	Precursor—LG	p.Q61R	2										**2**	1
107	F	61	Incidental	Precursor—LG			p.R201H	11								**11**	1

(Continued on the following page)

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Table 2. Somatic mutations detected in pancreatic juice by digital NGS (Cont'd)

					KRAS		*GNAS*		*RNF43*		*TP53*		*SMAD4*				
Case#	**M/F**	**Age**	**Risk**	**Study group**		**dNGS#**		**dNGS#**		**dNGS#**		**dNGS#**		**dNGS#**	**Other[a] dNGS#**	**Total dNGS#**	**# of unique mutations**
87	M	65	Incidental	Precursor—LG			p.R201H	2			p.R282Q	3			10	**15**	4
71	M	65	Familial	Precursor—LG	p.G12D/V/R G13R, Q61H	9,12,4,3,2	p.R201C/H	7,3	p.R132X	4						**44**	7
070[b]	M	62	Familial	Precursor—ERCP	p.G12D/V/R, Q61H[c]	72,13,175,14	p.R201C/H	6,17	p.E43fs, R145X, R330X, Q768fs	39,16,5,4	p.M237fs, G245S	11,7			4	**383**	13
79	M	57	MAS	Precursor—MAS	p.G12D/R, Q61H[b]/L	3,1,3,6	p.R201C	131			p.R248Q	3				**147**	6
73	F	66	Familial	Precursor—HG	p.G12V/R	1,1					p.R273C, H365fs	3,8	p.G299fs, Y434fs	6,10	8	**37**	8
74	F	65	HR—PJS	Precursor—HG	p.G12D/V/C	4,2,2			p.R113X	9						**17**	4
81	F	87	Incidental	Precursor—HG			p.R201C	127								**127**	1
98	M	77	Incidental	Precursor—HG	p.G12D/A, G13R,Q61R	2,2,3,2	p.R201C	4	p.R145X	5	p.R283H	4				**22**	7
78	F	75	PC	PC	p.G12D	7					p.W91X, V274A, N288D	5,5,4				**21**	4
80	F	78	PC	PC	p.G12V/R	2,1	p.R201C	2					p.G89fs	5	9	**19**	6
82	M	62	PC	PC	p.G12D	34					p.S315Y	17				**51**	2
83	F	72	PC	PC									p.D537G	4		**4**	1
84	M	72	PC	PC with IPMN			p.R201C	2								**2**	1
85	M	73	PC	PC	p.G12D	3					p.R248W, N310D	3,4				**10**	3
86	M	65	PC	PC	p.G12D/V/R	13,2,2	p.R201C	2	p.L341fs	6	p.S315Y	8				**33**	6
88	F	77	PC	PC	p.G12V/R	1,35					p.R273C	32			11	**79**	5
94	M	53	PC	PC	p.G12V	8					p.H193Y	6			35	**49**	3
97	M	51	PC	PC	p.G12V, Q61K	1,46			p.H350fs	4						**51**	3
101	M	85	PC	PC with IPMN	p.G12D	2										**2**	1
102	M	68	PC	PC							p.G266fs	5	p.L533R	4		**9**	2
103	M	61	PC	PC with IPMN	p.G12D	111	p.R201C	80			p.Q331fs	72			34	**297**	4
106	F	62	PC	PC	p.G12D/V/R	3,2,9					p.R175H, R248Q	4,4				**22**	5

Abbreviations: ERCP, endoscopic retrograde cholangiopancreatography; dNGS#, digital NGS score (mutation concentration); F, female; HG, high grade; HR, high risk; LG, low grade; M, male; MAS, McCune–Albright syndrome; PC, pancreatic cancer; Precursor, precursor by surgical pathology.

[a]Other mutations: case #109, *CDKN2A* p.G67S (score 4); case #131, *TGFBR2* p.F136fs (score 4); case #96, *FBWX7* Y545C (score 4); case #118, *ARID1A* 3199-2A>AG (score 4); case #119, *CDKN2A* p.F90L (score 4); case #087, *ARID1A* p.S513fs (score 10); case #070, ARID1A p.S1839fs (score 4); case #073, FBXW7 p.G687fs (score 4) and an ARID1A S1998P (score 4); case #080, FBXW7 p.L417X (score 5) and an ARID1A Q1835R (score 4); case #088, CDKN2A p.R128W (score 4) and FBXW7 p.G687fs (score 7); case #94, ARID1A p.S513fs (score 17), p.Q529fs (score 6), and p.H544fs (score 12); and case #103, CDKN2A p.D108V (score 34).

[b]Juice collected by ERCP.

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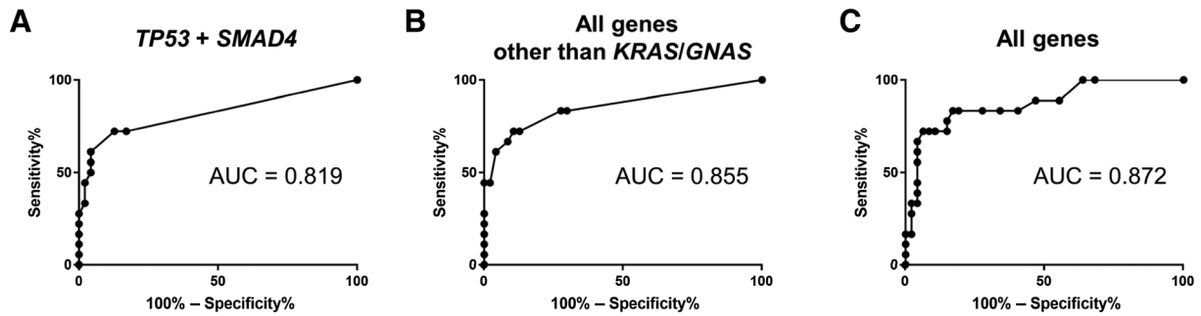

Figure 2.

ROC curve analysis of the diagnostic accuracy of pancreatic juice digital NGS scores. ROC curve analysis of the accuracy of the digital NGS score for predicting the presence or absence of pancreatic cancer or high-grade dysplasia using *TP53* or *SMAD4* mutations (**A**), all 9 genes apart from *KRAS* and *GNAS* (**B**), or all nine genes (*KRAS, GNAS, TP53, SMAD4, RNF43, CDKN2A, TGFBR2, ARID1A*, and *FBXW7*; **C**).

juice was also a predictor of neoplastic grade. Eleven of 18 patients with PDAC or high-grade dysplasia had two or more unique mutations other than *KRAS*/*GNAS* mutations compared with 5 of the remaining 47 subjects ($P = 0.0001$).

The cases that underwent pancreatic resection to manage suspicious lesions (the precursor group) were helpful in evaluating the potential accuracy of pancreatic juice mutation analysis for predicting neoplastic grade. Cases #77, #75, and #72 all had concerning lesions detected by imaging (solid lesions and dilated pancreatic duct), but surgical pathology determined these lesions as mainly related to focal (cases #77 and #75) or diffuse pancreatitis (case #72, with a germline *PRSS1* mutation), and their pancreatic juice mutation profiles were unremarkable. Three of the 4 cases (cases #76, #71, #87, and #107) that underwent pancreatic resection with larger and/or numerous IPMNs with PanINs without high-grade dysplasia had higher pancreatic juice mutation concentrations, but none had high *TP53*/*SMAD4* mutation scores.

In contrast, the 4 cases (cases #73, #74, #81, and #98) with high-grade dysplasia in their resection specimen all had high overall mutation scores in their pancreatic juice, and 2 of them had high *TP53*/*SMAD4* mutation scores (see also Tables 1 and 2). For example, case #73 proceeded to distal pancreatectomy for a 5-mm solid lesion in the tail seen by EUS and atypical cells on aspiration cytology. The patient also had numerous small pancreatic cysts. Pancreatic juice collected 17 months before surgery had multiple *TP53* and *SMAD4* mutations consistent with the high-grade dysplasia (PanIN-3) found in the resected pancreas.

Two cases with very high total digital NGS scores that were included had additional reasons besides neoplastic burden for their higher mutation concentrations. The first case was a patient with McCune–Albright syndrome due to having a postzygotic *GNAS* mutation (#79); these patients can have extensive IPMNs and indeed did have very high pancreatic juice *GNAS* R201C mutation concentrations, along with low concentrations of four unique *KRAS* mutations and one *TP53* mutation associated with evidence of extensive IPMNs. Three months after his juice collection, the patient underwent distal pancreatectomy for extensive IPMN with LGD. The second case with a very high digital NGS score was a high-risk individual (#70) who had juice collected from the main pancreatic duct by ERCP to evaluate a dilated pancreatic duct (6 mm). The higher juice mutation concentrations in pancreatic duct juice samples collected by pancreatic duct cannulation (~10× higher in prior studies; ref. 22) reflect the higher purity of pancreatic juice. The dilated pancreatic duct associated with two subcentimeter pancreatic cysts drove the decision to perform pancreatic resection. The resection specimen contained multiple PanIN-2 and incipient IPMN. The pancreatic juice mutation profile included high *KRAS* G12R (digital NGS score of 175) and *KRAS* G12D (score of 72) mutation concentrations; there were also lower concentrations of two other *KRAS* mutations, two *GNAS* mutations, four different *RNF43* mutations, one *ARID1A* mutation, and two different *TP53* mutations. These mutations likely came from both the incipient IPMN and the multiple PanIN-2 found in the resection specimen. The mutation profile is consistent with two larger mutant-*KRAS* clonal neoplasms as well as smaller clones harboring other *KRAS* or *GNAS* mutations. The lower *RNF43*, *ARID1A*, and *TP53* mutation concentrations (~10 lower concentrations relative to the dominant *KRAS* mutations) suggest the emergence of subclones of more advanced neoplastic grade. In contrast to the ~10-fold lower ratio of mutations in *TP53* and other genes in this case, the pancreatic juice mutation profiles from patients with pancreatic cancers generally had similar ratios of *KRAS* mutations to other mutations.

Discussion

The evaluation and management of the pancreas in patients undergoing screening and surveillance can be challenging. The main goal of pancreatic screening is to identify patients with advanced precancerous lesions and prevent the progression to invasive cancer by surgical resection. Most individuals undergoing pancreatic screening have only minor abnormalities detected by imaging and although few individuals progress to pancreatic cancer, imaging findings do not adequately predict those most likely to progress to invasive disease. Even among individuals in whom surgery is undertaken for concerning pancreatic imaging abnormalities, imaging does not adequately predict the grade of neoplasia. Decisions about optimal surveillance intervals or pancreatic resection often hinge on deciding the significance of imaging abnormalities that are not suspicious enough to clearly warrant surgical intervention. Patients with incidentally detected IPMN with worrisome findings that meet guideline criteria for resection often do not have advanced neoplasia at resection (34). Fukuoka guidelines developed for patients with sporadic

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pancreatic cysts are based on the natural history of patients with incidentally detected cysts and do not strictly apply to those with an inherited susceptibility to pancreatic neoplasia. Despite guidelines such as the Fukuoka guidelines (35, 36), patients will occasionally progress to advanced pancreatic cancer while under surveillance (13, 14, 19). The emergence of pancreatic cancer in high-risk individuals despite regular surveillance highlights several challenges. One challenge is identifying PanIN progression; most PanINs do not cause significant imaging abnormalities, and since subtle changes in the parenchyma surrounding PanIN are not specific for PanIN grade, imaging cannot detect progression of PanIN from low grade to high grade. This limitation is important because molecular evidence indicates that most pancreatic cancers arise from PanIN, not IPMN (37). This is thought to be true even for high-risk individuals who commonly have pancreatic cysts, many of which are IPMN, detected during screening (12). Another challenge is that even after an invasive pancreatic cancer develops, it may not be detectable by imaging until it forms a solid mass of approximately 5 mm in diameter. The time window for detecting such a mass before it progresses to a more advanced stage is thought to be rapid (16).

Pancreatic juice analysis has the potential to complement existing pancreatic imaging tests and to help evaluate the pancreas for PanIN and better predict the presence of advanced precancerous lesions. Overall, our results demonstrate the diagnostic potential of pancreatic juice mutation analysis. The number and concentration of different mutations, particularly those other than *KRAS* and *GNAS*, and especially *TP53*/*SMAD4* mutations, predicted the presence of pancreatic cancer or high-grade dysplasia with very good accuracy. These mutations were detected in the pancreatic juice of patients with pancreatic cancer in percentages (64% and 14% for *TP53* and *SMAD4*, respectively) similar to what is found in primary PDAC. Consistent with the molecular progression of pancreatic neoplasia, our pancreatic juice analysis found that the best predictors of having an invasive pancreatic cancer or a precursor lesion with high-grade dysplasia are the presence of *SMAD4* mutations, a high *SMAD4*/*TP53* mutation score, or a high overall mutation score. The *SMAD4*/*TP53* score had the highest specificity, whereas the total mutation score had the highest diagnostic sensitivity; a positive score for either one of these two scores had the highest overall diagnostic accuracy. An overall mutation score had somewhat higher diagnostic accuracy than the *SMAD4*/*TP53* mutation score, reflecting the limitations of using two genes to predict the grade of neoplasia.

Most of the cases with high-grade dysplasia were associated with IPMN; one case with PanIN-3 also had a high overall mutation score and a high *SMAD4*/*TP53* mutation score. Further study is needed to determine how well pancreatic juice analysis predicts the presence of PanIN-3 and how well pancreatic juice analysis can be used to predict the emergence of cancer from PanIN. Although small PanIN may not shed enough DNA to be detectable in juice samples, cases from this study and prior studies indicate that PanIN-associated mutations can be detected in pancreatic juice. Indeed, some patients have *KRAS* and sometimes other mutations detected in the pancreatic juice despite unremarkable pancreata by imaging (19, 23, 31, 38). Similarly, the higher pancreatic juice mutation scores than predicted by the number and size of their pancreatic cysts could have extensive PanIN not detectable by pancreatic imaging.

Perhaps the most likely setting where pancreatic juice analysis would be of value is for patients under surveillance who are being considered for pancreatic resection because of pancreatic imaging abnormalities that suggest progression to cancer or high-grade dysplasia. Of the 13 cases that went to surgery for suspected precancerous disease that had pancreatic juice analysis, the 5 cases with the lowest grade of precancerous pathology had either no mutations or only *KRAS*/*GNAS* mutations in their pancreatic juice. Two of these cases underwent surgery for very small mass-like lesions that turned out to be related to chronic pancreatitis; 1 case was a patient who had a germline *PRSS1* mutation and chronic pancreatitis and a small IPMN; and the other 2 cases had small IPMNs with low-grade dysplasia, one of which involved the main pancreatic duct. The pancreatic juice mutation results in these cases predicted that they likely did not have high-grade dysplasia or invasive cancer, and so they could have continued surveillance instead of undergoing surgery without much risk of progressing to cancer in the near future. Improvements in preoperative pancreatic evaluation with pancreatic juice mutation analysis and other tests might help minimize unnecessary surgical resections for patients with indeterminate lesions detected by imaging.

Many pancreatic juice samples have low digital NGS mutation scores reflecting low mutation concentrations. Variable mutant DNA concentrations in different patients with pancreatic cancer may reflect differences in the extent of tumor DNA shedding into pancreatic juice relative to levels of wild-type DNA. Collecting pure samples of pancreatic juice [which have much higher mutation concentrations (22)] from the pancreatic duct would improve the detection of mutations and likely the overall diagnostic accuracy of tests applied to these samples, but ERCP sampling of pancreatic juice is too invasive for routine surveillance. It is possible that other pancreatic juice biomarkers of neoplasia could be combined with mutations to improve diagnostic accuracy (39). Further experience using pancreatic juice mutation tests are needed to determine how best to use this test to improve the management of patients under surveillance. A pancreatic juice test is likely to be most useful when used with pancreatic imaging to improve the prediction of the extent and grade of neoplasia. Although pancreatic imaging is imperfect, it is still the main test used to guide decisions about pancreatic surgery. Pancreatic juice analysis will likely to be most useful in helping to determine optimal surveillance intervals; concerning pancreatic juice mutation profiles could also indicate the need for complementary pancreatic testing. A pancreatic juice test would complement pancreatic cyst fluid tests, which are most helpful in evaluating the neoplastic nature and grade of cysts with worrisome features (40–43), but not for evaluating numerous small cysts or for detecting molecular evidence of occult neoplasia. It is also possible that the absence of mutations in pancreatic juice would better predict the risk of neoplastic progression in the future compared with imaging alone, but further study is needed to determine this. An important question yet to be determined is the likelihood of future neoplastic progression in patients who have pancreatic juice mutations that indicate more than low-grade dysplasia.

Pancreatic juice analysis could potentially provide complementary results to mutational analysis of fine-needle aspirates or biopsies, particularly since tumor heterogeneity and sampling can limit the yield of mutations detected in these samples (44). If so, it could be used to help identify actionable mutations for

targeted therapy, for example, *BRCA/PALB2/ARID1A* mutations and PARP inhibitors (45–49). Further study would be needed to address this question.

Several NGS methods are being evaluated for the detection of low-abundance mutations (44, 50). It is not yet known which method is the most accurate; employing the most accurate methods for detecting low-abundant mutations could improve the diagnostic yield of pancreatic juice analysis.

In summary, we find digital NGS analysis of pancreatic juice collected during EUS evaluation can help predict the presence of cancer and the grade of pancreatic dysplasia. Further evaluation using pancreatic juice mutation analysis as a clinical test in a prospective trial is warranted.

Disclosure of Potential Conflicts of Interest

E.-J. Shin is a consultant/advisory board member for Boston Scientific and C2 Therapeutics. M.I. Canto reports receiving commercial research grants from C2 Therapeutics and is a consultant/advisory board member for Pentax. No potential conflicts of interest were disclosed by the other authors.

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