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KRAS and *GNAS* Mutations in Pancreatic Juice Collected From the Duodenum of Patients at High Risk for Neoplasia Undergoing Endoscopic Ultrasound

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Abstract

BACKGROUND & AIMS—Pancreatic imaging can identify neoplastic cysts but not microscopic neoplasms. Mutation analysis of pancreatic fluid following secretin stimulation might identify microscopic neoplasias in the pancreatic duct system. We determined the prevalence of mutations in *KRAS* and *GNAS* genes in pancreatic juice from subjects undergoing endoscopic ultrasound for suspected pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasms, or pancreatic adenocarcinoma.

METHODS—Secretin-stimulated juice samples were collected from the duodenum of 272 subjects enrolled in Cancer of the Pancreas Screening studies; 194 subjects were screened because of a family history of, or genetic predisposition to, pancreatic cancer and 78 were evaluated for pancreatic cancer (n=30) or other disorders (controls: pancreatic cysts, pancreatitis, or normal pancreata, n=48). Mutations were detected by digital high-resolution melt-curve analysis and pyrosequencing. The number of replicates containing a mutation determined the mutation score.

RESULTS—*KRAS* mutations were detected in pancreatic juice from larger percentages of subjects with pancreatic cancer (73%) or undergoing cancer screening (50%) than controls (19%) (*P*=.0005). A greater proportion of patients with pancreatic cancer had at least 1 *KRAS* mutation detected 3 or more times (47%) than screened subjects (21%) or controls (6%, *P*=.002). Among screened subjects, mutations in *KRAS* (but not *GNAS*) were found in similar percentages of patients with or without pancreatic cysts. However, a greater proportion of patients over 50 ys old had *KRAS* mutations (54.6%) than younger patients (36.3%) (*P*=.032); the older subjects also more mutations in *KRAS* (*P*=.02).

CONCLUSIONS—Mutations in *KRAS* are detected in pancreatic juice from the duodenum of 73% of patients with pancreatic cancer, and 50% of asymptomatic individuals with a high risk for pancreatic cancer. However, *KRAS* mutations are detected in pancreatic juice from 19% of controls. Mutations detected in individuals without pancreatic abnormalities, based on imaging analyses, likely arise from small PanIN lesions. ClinicalTrials.gov no: NCT00438906 and NCT00714701

Keywords

screening; EUS; early detection; IPMN; PDAC

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States and is increasing in incidence.[1] Most patients with ductal adenocarcinoma of the pancreas are diagnosed at an advanced stage of disease. Approximately 15% of patients who present with symptoms from their cancer have a resectable cancer; and of these, only ~10% have stage 1 disease.[2] Patients who present with low-stage cancers have a better outcome than patients who present with advanced disease. Effective pancreatic screening strategies are needed to improve the detection of low-stage asymptomatic pancreatic cancer and its precursors.

The most common precursors to pancreatic adenocarcinoma are pancreatic intraepithelial neoplasias (PanINs) and the cyst-forming lesions intraductal papillary mucinous neoplasms

(IPMNs) and mucinous cystic neoplasms (MCNs). PanINs are microscopic lesions usually <5 mm, while IPMNs and MCNs are larger (>1 cm), grossly visible lesions[3]. Low-grade PanIN-1 lesions are found in the majority of pancreata of middle-aged patients.[4, 5] In contrast, PanIN-3 lesions (high-grade dysplasia) are usually found in the pancreata of patients with invasive pancreatic cancer and in subjects with a strong family history of pancreatic cancer.[6, 7] IPMNs are also prevalent and can be detected incidentally in ~2–10% of older adults,[8, 9] and are also more prevalent among subjects with a strong family history of pancreatic cancer.[10] Pancreatic screening may be appropriate for individuals at sufficiently increased risk of developing pancreatic cancer.[10] The primary goal of pancreatic screening is to identify these potentially curable non-invasive precursor neoplasms, particularly high-grade precursor lesions, and the smallest earliest-stage pancreatic cancers.

EUS and MRI/MRCP are effective pancreatic screening tests, particularly for identifying very small pancreatic cysts[10], and EUS is a useful tool for detecting small pancreatic cancers.[11] For these reasons, and because these modalities do not expose patients to radiation, EUS and MRI/MRCP are considered to be the best available pancreatic screening tests. Since these tests cannot diagnose PanIN lesions[12], novel approaches are needed to identify these small lesions. One diagnostic approach being evaluated for its potential to detect pancreatic neoplasia is the analysis of pancreatic juice for genetic mutations. This approach utilizes our knowledge of the genetic alterations in precursor lesions and the fact that most pancreatic precursor lesions arise within the duct system. For example, taking advantage of the very high specificity of mutant *GNAS* as a marker of IPMNs[13], we recently found that the prevalence of mutant *GNAS* in duodenal collections of secretin-stimulated pancreatic juice from patients with IPMNs is similar to that found in resected IPMNs[14]. We also showed that the detection of *TP53* mutations in secretin-stimulated pancreatic juice samples is a highly specific marker of invasive pancreatic cancer and high-grade dysplasia.[15] Some patients with *TP53* mutations in their pancreatic juice appeared to have PanIN-3 lesions as the source of their mutation. Although these results highlight the potential power of this approach, further studies are needed before they can be applied clinically.[16]

Oncogenic *KRAS* mutations are found in >90% of PanINs[1718] and in the majority of IPMNs and MCNs.[13, 19] Although several studies that have evaluated the diagnostic utility of using mutant *KRAS* have found it to be a useful marker for evaluating focal pancreatic lesions such as masses or cysts[13, 20], *KRAS* mutations are not specific for high-risk lesions. They are also very prevalent in low-grade PanINs and low-grade IPMNs[1718], and most of these low-grade lesions do not progress to invasive carcinoma.[21] Indeed, studies evaluating oncogenic *KRAS* mutations detected in pancreatic juice sampled from the pancreatic duct have found that mutant *KRAS* is often detected in patients without pancreatic cancer or high-grade pancreatic precursor neoplasms.[22–25]

In this study, we used digital high-resolution melt-curve analysis (digital-HRM) and pyrosequencing to measure *KRAS* and *GNAS* mutation concentrations in secretin-stimulated duodenal collections of pancreatic juice from individuals undergoing pancreatic evaluation performed as part of the CAPS studies.[10, 26]

MATERIALS AND METHODS

All elements of this investigation have been approved by The Johns Hopkins Medical Institutional Review Board (IRB) and the IRBs of each participating site. Written informed consent was obtained from all patients. All authors reviewed the manuscript and agreed to its submission.

Patients and Specimens

Pancreatic juice samples and subject data for this study were obtained from 272 study subjects enrolled in the CAPS studies.[10, 26] Most subjects (n=240) in this study participated in the multi-center CAPS3 study (2007–2009) (clinicaltrials.gov: NCT00438906), further described elsewhere.[10] To increase the number of disease controls, 32 subjects from CAPS2[26] and CAPS4 (NCT00714701) were also included in this study. The final study population included 194 patients who underwent pancreatic screening and 78 patients evaluated for other indications.

The 194 subjects who underwent pancreatic screening were asymptomatic individuals who met the appropriate age criteria with either (i) a strong family history of pancreatic cancer (with at least one affected first-degree, and one affected second-degree relative with pancreatic cancer); (ii) germline mutation carriers (*BRCA2, p16, BRCA1*, HNPCC genes) with a family history of pancreatic cancer or (iii) Peutz-Jeghers syndrome.

Seventy-eight of the 272 individuals enrolled in the CAPS studies underwent EUS for suspected pancreatic disease or for other indications. These included (i) 30 patients with symptomatic pancreatic ductal adenocarcinoma (10 with resectable cancers, 20 with locally advanced and/or metastatic disease), (ii) patients with a pancreatic cyst (suspected IPMN) without a family history of pancreatic cancer (n=17), (iii) patients with clinical features of chronic pancreatitis confirmed by pancreatic imaging (n=9), and (iv) patients with no evidence of pancreatic exocrine disease after pancreatic evaluation (normal pancreas controls, n=22) (Supplemental Table 1).

Pancreatic juice secretion was stimulated by infusing human synthetic secretin (ChiRhoClin, Inc, Burtonsville, MD) (0.2 ug/kg/i.v. over a minute), then collected from the duodenal lumen for ~5 minutes (typically, 5–10 mls), as it was secreted from the ampulla by suctioning fluid directly into the echoendoscope channel without the use of a catheter.[14]

KRAS and GNAS mutation detection

High-resolution digital HRM analysis was performed as previously described.[17] The number of PCR reactions containing a mutation determined the mutation score. Higher mutation scores (3 or more, ~0.1% mutation concentration) best separated pancreatic cancer cases from other patient groups. Details of the methods are provided in Supplemental materials.

Statistical Analysis

Mean mutation scores in groups were compared by Mann-Whitney's U-test or Student t-test. ANOVA was used to evaluate associations between clinical factors and mutation score. Chi-square analysis was used to compare the proportions of subjects in each group. Statistical

analysis was performed using SPSS Statistics 17.0 software (IL, USA). $P<0.05$ was considered statistically significant.

RESULTS

Detection of mutant KRAS in Pancreatic Juice Collected from the Duodenum

A summary of the characteristics of the 272 individuals in our study is presented in Table 1 and Supplemental Table 1. Subjects with pancreatic cancer were older than disease controls (mean/s.d., age 62.2+/−10.3 vs. 51.3+/−13.2 years, p=0.0001, Student t-test), and the 76 subjects who underwent screening and found to have a pancreatic cyst were older than the 118 who underwent screening and not found to have a cyst (59.4+/−9.0 vs. 53.6+/−10.4 years, P<0.0001) (Table 2).

KRAS mutations were detected significantly more often in juice samples from patients with pancreatic cancer (22/30, 73.3%), and subjects undergoing screening (96/194, 50%), than in other patients (12/48, 25%, p<0.0005). Patients with pancreatic cancer (n=30) were also more likely to have detectable *KRAS* mutations in their duodenal fluid than the 48 disease controls without pancreatic cancer (who had either chronic pancreatitis, a sporadic pancreatic cyst or a normal pancreas) (73.3% vs. 25%, p=0.00001), as well as the 194 patients undergoing pancreatic screening for their familial/genetic predisposition to pancreatic cancer (49.5%, p=0.015). Patients with pancreatic cancer (n=30) were also more likely to have higher concentrations of *KRAS* mutations, reflected by mutation scores of ≥3 compared to all other groups (n=242) (50% vs. 17.4%, p=0.0005), including the 48 disease controls (10.4%, p<0.0001) and the 194 patients undergoing screening (18.6%, p<0.0001). Patients undergoing pancreatic screening (n=194) were also more likely to have detectable *KRAS* mutations than the 48 disease controls without pancreatic cancer (49.5% vs. 25%, p=0.002). Among the 194 subjects undergoing pancreatic screening, there was no difference in the prevalence of *KRAS* mutations among subjects with (n=76) vs. those without (n=118) pancreatic cysts (Table 2).

Mean duodenal fluid concentrations of mutant *KRAS* were also significantly higher in the 30 patients with pancreatic cancer than the 48 disease controls (mean score/s.d./range; 6.1/7.2/0–26 vs. 1.3/3.4/0–5, p=0.02), and the 194 patients undergoing screening (1.9/3.8/0–31, p<0.01) (Table 1). We also evaluated the diagnostic utility of using the concentration of the dominant *KRAS* mutation as a way of classifying groups. We found that the 30 patients with pancreatic cancer were more likely to have an individual *KRAS* mutation with a score of ≥3 (47%) than all other patient groups including patients undergoing screening (39/194, 20.1%), and disease controls (one with chronic pancreatitis, one with sporadic pancreatic cyst and one normal pancreas control) (6%, p=0.002). Patients in the screening group with pancreatic cysts (n=76) were not more likely to have a total *KRAS* mutation score of ≥3 than patients in the screening group without cysts (n=118, p=0.09), but were more likely to have an individual *KRAS* mutation with a score of ≥3 (11.8% vs. 4.2%, p=0.05).

The 76 patients who underwent pancreatic screening and were found to have pancreatic cysts were also more likely to have *KRAS* mutations detected in their pancreatic fluid than

were the 17 individuals with sporadic pancreatic cysts (p=0.047), and the 22 individuals with a normal pancreas (p=0.008).

The most common *KRAS* gene mutations identified were the G12D, G12V and G12R mutations, which are also the most common mutations found in pancreatic cancers, PanINs and IPMNs (Table 4).[17, 20, 27]

We examined if age, gender, smoking status, and body mass index (BMI) were associated with the detection of *KRAS* mutations among the 194 subjects undergoing screening. The average age of the 96 subjects with a *KRAS* mutation in their duodenal fluid was significantly higher than the average age of the 98 individuals without *KRAS* mutations (57.6+/−9.6 vs. 54.1+/−10.6 years, p=0.019). The prevalence of *KRAS* mutations was also significantly lower in subjects under the age 50 than it was in subjects 50 years of age or older (p<0.02). We also found that *KRAS* mutation concentrations among the 194 subjects undergoing screening were higher in those over 50 than it was in those under 50 (p<0.016). We did not find any association between gender, smoking status, or BMI and the prevalence of *KRAS* mutations (Table 5).

Relationship between mutant KRAS and mutant GNAS in pancreatic juice samples

KRAS mutations were detected more often among the 194 patients undergoing pancreatic screening than were *GNAS* mutations (49.5% vs. 19.2%, p<0.0001) (Table 5). We previously reported that pancreatic fluid *GNAS* mutations were a highly specific marker for the presence of pancreatic cysts.[14] Among the 76 patients undergoing screening who were found to have pancreatic cysts, *GNAS* mutations were detected in 51.5% of juice samples compared to 49% for *KRAS* mutations. We did not find a higher prevalence of *KRAS* mutations in the pancreatic juice of subjects who also had detectable *GNAS* mutations compared to those without *GNAS* mutations (data not shown). *KRAS* mutations were also observed more often in patients under age 50 (20/52) than were *GNAS* mutations (4/52 cases, p<0.0002) (Table 5).

Additional results are available in Supplemental materials.

Discussion

We found that *KRAS* mutations could be detected in secretin-stimulated pancreatic juice collected from the duodenum of patients with and without significant pancreatic disease, and that these mutations have only a modest ability to discriminate pancreatic cancer cases from other diagnostic groups. In fact, we find low concentrations of *KRAS* mutations (mutation scores <3) are often present in pancreatic juice collected from the duodenum of patients without any imaging evidence of pancreatic disease. Patients with pancreatic cancer were significantly more likely to harbor *KRAS* mutations and to have higher *KRAS* mutation concentrations (mutation scores ≥3) than other patient groups. Furthermore, patients with pancreatic cancer were significantly more likely to have one or more *KRAS* mutations with scores of ≥3 in their pancreatic fluid than patients undergoing screening and disease controls (47.3% vs. 20.1% vs. 6.2%) (Table 1). But while high *KRAS* mutation scores had some ability to distinguish pancreatic cancer cases from other patient groups, overall, our results

indicate that mutant *KRAS* measurements in pancreatic fluid cannot be used as a single marker to reliably distinguish patients with pancreatic cancer or pancreatic cysts from those without clinical evidence of pancreatic neoplasia.

Patients with pancreatic cancer have much higher *KRAS* mutation concentrations in pancreatic juice samples collected from the pancreatic duct compared to the duodenal lumen.[22] When secretin-stimulated pancreatic fluid collects in the duodenal lumen, it mixes with DNA in the duodenal lumen and dilutes pancreatic fluid DNA. Many patients with advanced pancreatic cancer have pancreatic insufficiency[28] and as a result can have reduced pancreatic fluid secreted after secretin-stimulation. Lower ratios of pancreatic fluid/duodenal fluid DNA in duodenal collections after secretin stimulation in some patients with advanced pancreatic cancer may result in reduced concentrations of pancreatic fluid mutations in duodenal fluid samples. Indeed, in a study of patients who had secretin-stimulated pancreatic fluid collected from the duodenum during EUS and the pancreatic duct at later ERCP, low relative concentrations of pancreatic-to-duodenal fluid DNA was associated with reduced detection of mutations in duodenal fluid.[29] It therefore may not be surprising that the concentration of *KRAS* mutations in pancreatic juice samples from patients with advanced pancreatic cancer was only modestly higher (~5–10 fold) than the concentration in samples from those without detectable pancreatic neoplasia. Pancreatic insufficiency is unlikely to be present in patients with small low-stage pancreatic cancers so may not be a significant factor for patients with pancreatic neoplasia undergoing pancreatic screening. There are likely to be other factors that influence the concentration of pancreatic fluid mutations in patients with pancreatic cancer. For example, it is possible that only a small portion of an infiltrating pancreatic cancer sheds DNA into the pancreatic ducts and from there into the pancreatic juice; portions of the cancer may obstruct draining pancreatic ductules and, as a result, much of the cancer may be isolated from the main pancreatic ductal system by the fibro-inflammatory response to the infiltrating cancer.

Interestingly, many patients undergoing pancreatic screening with normal-appearing pancreata had detectable *KRAS* mutations in their pancreatic juice. The only risk factor associated with the detection of pancreatic juice *KRAS* mutations in the high-risk group was age, with subjects over 50 years of age being more likely to harbor a *KRAS* mutation than those younger than 50. This observation may be explained by the presence of multifocal PanIN lesions too small to detect by imaging. Previous autopsy studies revealed that most adults harbor PanIN-1 lesions, which increase in prevalence with age, particularly among individuals over 50 years of age.[5] Since over 90% of PanIN-1 lesions harbor mutant *KRAS*[17], the high prevalence of *KRAS* mutations in pancreatic juice is consistent with the hypothesis that most *KRAS* mutations detected in pancreatic juice samples arise from microscopic PanIN lesions. For this reason, unlike *GNAS* mutations, *KRAS* mutations detected in pancreatic juice were not found to be a specific marker of the presence of a pancreatic cyst. This is not surprising since subjects undergoing pancreatic screening can harbor PanIN whether or not they have pancreatic cysts. Interestingly, we found that subjects undergoing screening with pancreatic cysts were more likely to have an individual *KRAS* mutation above a certain cut-off than those without a cyst, although overall mean *KRAS* mutation concentrations were not significantly different. Most pancreatic cysts

identified in patients undergoing pancreatic screening are thought to be either IPMNs or incipient IPMNs because their juice samples often contain mutant *GNAS*[14, 30], and over time even small subcentimeter cysts can progress to larger cysts with typical characteristics of IPMNs.[7, 10, 26] However, even among patients who undergo pancreatic resection for an IPMN identified by screening, PanIN lesions are usually considerably more numerous than IPMN lesions.[6, 7] Although the average size of pancreatic cysts in our screening group was significantly smaller than the cysts from sporadic patients, their duodenal fluid *KRAS* mutation concentrations were significantly higher. This supports the hypothesis that the amount of mutant *KRAS* shed into the pancreatic juice by pancreatic cyst(s) does not dominate the contribution provided by multifocal PanIN lesions.

Overall, our results suggest that mutant *KRAS* detected in pancreatic juice samples should not be considered a specific marker of pancreatic cancer or of a macroscopic pancreatic cyst. In most individuals, the presence of mutant *KRAS* may simply reflect the presence of PanINs lesions that are usually multifocal and of low neoplastic grade. The presence of higher concentrations of mutant *KRAS* (mutation scores ≥3) likely indicates more extensive pancreatic neoplasia than does lower mutation scores, but *KRAS* mutation scores alone cannot reliably distinguish the presence of cancer from low-grade dysplasia. The inability of pancreatic juice *KRAS* mutations to reliably distinguish low-grade precursors from high-grade dysplasias and invasive cancers is in contrast to other mutations, such as *GNAS* which is more specific for IPMNs, and mutant *TP53,* which is not found in low-grade PanINs or in low-grade IPMNs but often present in high-grade dysplasias and invasive cancers.[15] The results of pancreatic fluid analysis, which provide a sample of the whole pancreatic duct system, are also in contrast to directed FNA sampling of focal lesions such as pancreatic cysts, as studies have found that mutant *KRAS* and *GNAS* detected in cyst fluid is a useful marker for distinguishing mucinous from non-mucinous pancreatic cysts[13, 20]

In conclusion, we find that *KRAS* mutations are commonly detected in the duodenal fluid of patients with pancreatic cancer and patients undergoing pancreatic screening, including high-risk subjects with a normal appearing pancreas on imaging. Low concentrations of *KRAS* mutations are also often detected in the fluid of patients not suspected to have pancreatic disease. Our results highlight the need for more specific pancreatic juice markers of high-grade dysplasia and invasive pancreatic cancer.

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
s.d	standard deviation
HNPCC	hereditary non-polyposis colorectal cancer

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Table 1

Patient characteristics and Pancreatic juice KRAS mutations

	Screened individuals (family history/gene mutation	PDAC	sporadic pancreatic cyst(s)	chronic pancreatitis	normal pancreas	*p-value*
n	194	30	17	9	22	
Gender (Male/Female)	92/102	12/18	9/8	5/4	12/10	*0.82*
Age (mean/SD)	55.9/10.2	62.2/10.3	66.6/10.0	49.1/11.0	51.8/13.3	*<0.0001**
Race (Caucasian/non-Caucacian)	191/3	22/8	14/3	8/1	16/6	*<0.0001**
Smoking history	72/174 (41.4%)	13/24 (54.2%)	5/14 (35.7%)	1/9 (11.1%)	7/20 (35.0%)	*0.24*
Brinkman index (mean/SD)	121.8/250.2	136.6/221.3	159.9/322.3	55.6/75.9	118.0/50.9	*0.40*
BMI (mean/SD)	27.7/5.0 (n=127)	25.6/4.5 (n=15)	27.3/5.3 (n=12)	26.3/3.1 (n=6)	29.7/7.2 (n=8)	*0.36*
Cyst size (mm) (mean/SD)	-	-	21.6/12.4	-	-	
Number of the cysts (mean/SD)	-	-	2.4/2.6	-	-	
Prevalence of mutant KRAS	49.5%	73.3%	23.5%	44.4%	18.2%	*0.0005**
Total KRAS mutation score (mean/SD)	1.9/3.8	6.1/7.2	0.5/0.9	1.1/1.8	0.6/1.4	*<0.0001**
Total KRAS mutation score ≥3	18.6%	50.0%	5.9%	22.2%	9.1%	*0.0005**
Individual KRAS mutation score ≥3	20.6%	46.7%	5.9%	11.1%	4.5%	*0.0024**

Table 2

Patient characteristics and Pancreatic juice KRAS mutations in screened individuals

	Screening individuals (total 194)		
	Pancreatic cysts	**No cysts**	***p-value***
n	76	118	
Gender (Male/Female)	36/40	56/62	*0.99*
Age (mean/SD)	59.4/9.0	53.6/10.4	*<0.0001**
Race (Caucasian/non-Caucacian)	75/1	116/2	*0.69*
Smoking history	33/69 (47.8%)	39/105 (37.1%)	*0.21*
Brinkman index (mean/SD)	171.2/305.8	88.8/199.8	*0.16*
BMI (mean/SD)	27.8/5.4 (n=50)	27.6/4.8 (n=77)	*0.92*
Cyst size (mm) (mean/SD)	6.2/3.8	-	
Number of the cysts (mean/SD)	2.9/2.5	-	
Prevalence of mutant KRAS	48.7%	50.0%	*0.86*
Total KRAS mutation score (mean/SD)	2.5/5.0	1.5/2.8	*0.50*
Total KRAS mutation score ≥3	25.0%	15.3%	0.09
Individual KRAS mutation score ≥3	27.6%	16.1%	0.05

Table 3

Characteristics of subjects undergoing screening by pancreatic juice KRAS status

	Wild type	**KRAS mutant**	***p-value***
n	98	96	
Gender (Male/Female)	45/53	47/49	*0.67*
Age	55.2/11.2	56.6/9.1	*0.25*
Age ≥50	65.3%	78.1%	*0.047**
Risk factor (familial/Germline)	88/10	89/7	*0.61*
Smoking history (Y/total)	35/88 (39.7%)	37/86 (43.0%)	*0.66*
Brinkman index (mean/SD)	145.5/303.7	97.6/178.6	*0.88*
BMI	27.2/4.6 (n=61)	28.1/5.4 (n=66)	*0.32*
Cyst status			
Pancreatic cysts	39	37	*0.86*
No cysts	59	59	

Table 4

Percentages of each pancreatic juice KRAS mutation detected

KRAS mutation type	number of mutations detected in juice samples	% of total mutations detected in juice samples	% of juice samples containing mutation
G12D	299	38%	36.70%
G12V	220	28.20%	28%
G12R	188	24.10%	36.00%
G12A	45	5.80%	17.30%
G12C	27	0.35%	3%

Table 5

Characteristics of patients undergoing screening stratified by age

	age <50	**age >50**	***p-value***
n	55	139	
Gender (Male/Female)	26/29	66/73	*0.98*
Age (mean/SD)	44.0/5.0	60.6/7.6	*<0.0001**
Diagnosis			
Pancreatic cyst	8	68	*<0.0001**
No cysts	47	71	
KRAS mutation prevalence	38.2%	54.0%	*0.048**
KRAS mutation score (mean/SD)	1.0/2.7	2.2/4.2	*0.01**
GNAS analysis	n=52	n=130	
GNAS mutation (Y/N)	4/48	35/95	*0.0043**
GNAS mutation score (mean/SD)	0.34/1.4	1.82/3.90	*0.0038**
KRAS/GNAS mutation			
MT/MT	1	15	*0.006 **
MT/WT	19	54	
WT/MT	3	20	
WT/WT	29	41	