Published in final edited form as:
*Gut*. 2013 July ; 62(7): 1024–1033. doi:10.1136/gutjnl-2012-302823.

# Mutant *GNAS* detected in duodenal collections of secretin-stimulated pancreatic juice indicates the presence or emergence of pancreatic cysts

**Mitsuro Kanda**[1], **Spencer Knight**[1], **Mark Topazian**[5], **Sapna Syngal**[6], **James Farrell**[7], **Jeffrey Lee**[8], **Ihab Kamel**[4], **Anne Marie Lennon**[2], **Michael Borges**[1], **Angela Young**[1], **Sho Fujiwara**[1], **Junro Seike**[1], **James Eshleman**[1,3], **Ralph H. Hruban**[1,3], **Marcia Irene Canto**[1,2], and **Michael Goggins**[1,2,3]

[1]Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institutions

[2]Department of Medicine, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institutions

[3]Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institutions

[4]Department of Radiology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institutions

[5]Mayo Clinic

[6]Dana Farber Cancer Institute

[7]University of California at Los Angeles

[8]MD Anderson Cancer Center

## Abstract

**Objective—**Pancreatic cysts are commonly detected in patients undergoing pancreatic imaging. Better approaches are needed to characterize these lesions. In this study we evaluated the utility of detecting mutant DNA in secretin-stimulated pancreatic juice.

**Design—**Secretin-stimulated pancreatic juice was collected from the duodenum of 291 subjects enrolled in Cancer of the Pancreas Screening trials at 5 US academic medical centers. The study

Send correspondence to: Michael Goggins, MD, Johns Hopkins Medical Institutions, Department of Pathology, 1550 Orleans Street, Baltimore, MD 21231. Tel: 410 955 3511, Fax: 410 614 0671, mgoggins@jhmi.edu.

Disclosures: Drs. Goggins and Hruban have a licensing agreement with Myriad Genetics for the discovery of *PALB2* as a pancreatic cancer susceptibility gene. There are no other conflicts of interest for any of the authors. Recombinant secretin was provided for this study but the companies involved had no part in the design of this study, analysis or interpretation of data or in the writing of this manuscript. The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and statistical analysis. "The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Gut editions and any other BMJPGL products to exploit all subsidiary rights, as set out in our licence"

Author contributions: Mitsuro Kanda; acquisition of data; analysis and interpretation of data; drafting of the manuscript. Spencer Knight; Michael Borges; Angela Young, Sho Fujiwara, Junro Seike; acquisition of data James Eshleman, Mark Topazian, Sapna Syngal, James Farrell, Jeffrey Lee, Ihab Kamel, Anne Marie Lennon; generation of data, revision of the manuscript. Ralph H. Hruban; material support; acquisition and interpretation of data; manuscript revision. Marcia I. Canto; material support; acquisition and interpretation of data; study supervision; revision of the manuscript, obtained funding. Michael Goggins; study concept and design; study supervision; interpretation of data; drafting of the manuscript; obtained funding

population included subjects with a familial predisposition to pancreatic cancer who underwent pancreatic screening, and disease controls with normal pancreata, chronic pancreatitis, sporadic IPMN, or other neoplasms. Somatic *GNAS* mutations (reported prevalence; ~66% of IPMNs) were measured using high-resolution digital melt-curve analysis and pyrosequencing.

**Results—***GNAS* mutations were detected in secretin-stimulated pancreatic juice samples of 50 of 78 familial and sporadic cases with IPMN(s) (64.1%), 15 of 33 (45.5%) with only diminutive cysts (<5mm), but none of 57 disease controls. *GNAS* mutations were also detected in 5 of 123 screened subjects without a pancreatic cyst. Among 97 subjects who had serial pancreatic evaluations, *GNAS* mutations detected in baseline juice samples predicted subsequent emergence or increasing size of pancreatic cysts.

**Conclusion—**Duodenal collections of secretin-stimulated pancreatic juice from patients with IPMNs have a similar prevalence of mutant *GNAS* to primary IPMNs, indicating these samples are an excellent source of mutant DNA from the pancreas. The detection of *GNAS* mutations before an IPMN is visible suggests that pancreatic juice analysis has potential to help in the risk stratification and surveillance of patients undergoing pancreatic screening.

### Keywords

Pancreatic cancer; intraductal papillary mucinous neoplasm; pancreatic cyst; pancreatic juice; GNAS

## INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States[1]. Most patients with pancreatic ductal adenocarcinoma present with advanced-stage disease, with a 5-year survival rate of <5%[2]. In contrast, resection of precursor neoplasms can prevent the development of pancreatic ductal adenocarcinoma[3]. PanINs are considered the commonest precursor to pancreatic adenocarcinoma. PanINs are microscopic lesions (by definition <1mm to ~5mm diameter), generally too small to be identified by current imaging tests. IPMNs are larger cystic pancreatic precursor neoplasms[4] with an estimated prevalence of ~2% of older adults[5,6]. The primary goal of pancreatic screening is to identify and treat these potentially-curable non-invasive precursors before they evolve into incurable invasive pancreatic adenocarcinomas[7]. Pancreatic screening of individuals with a strong family history of pancreatic cancer using pancreatic imaging tests (EUS and/or MRI/MRCP) detects a high prevalence of pancreatic cysts, many of which are IPMNs[8–15].

In the Cancer of the Pancreas Screening-3 (CAPS3) study, we found 84/216 (38%) of subjects screened had pancreatic cysts, (mean size 0.55 cm, range 2–39 mm). Cysts were detected more often in older subjects: (14% of subjects age <50, 35% aged 50–59, and 62% aged 60–69 years, p<.0001)[8]. Subcentimeter pancreatic cysts are usually not resected so information about their pathology is lacking. In addition, resected pancreata of screened individuals typically contain multifocal PanIN, in addition to IPMNs[16]. Extensive PanINs may produce gland heterogeneity visible by EUS, but such changes are subtle and non-specific[17]. Thus, PanINs are only identified after histological examination of the resected pancreas. The inability to identify PanINs preoperatively and our inability to characterize small pancreatic cysts highlight a need for novel diagnostic approaches to better characterize these lesions. One promising approach is to analyze pancreatic juice for mutations arising from the pancreas, a so-called “endoscopic pancreatic juice DNA test”.

Several studies have evaluated the diagnostic potential of pancreatic juice markers[18–23], but these studies have usually analyzed pure pancreatic juice collected from the pancreatic duct during or after ERCP[24]. However, pancreatic duct sampling is too invasive for screening.

Duodenal collections of pancreatic juice collected during upper endoscopy after secretin infusion are used to evaluate pancreatic function as a test of pancreatic insufficiency[25], but this sample has not been evaluated as a source of mutant DNA. Pancreatic juice could be a rich source of pancreatic markers and when collected from the duodenum without directly sampling the pancreas could be a safer sample to obtain than fine needle aspirates[26]. Pancreatic marker concentrations are lower in duodenal collections of pancreatic juice than in FNAs or pure pancreatic juice collections, necessitating the use of accurate markers and sensitive assays. Mutant DNA can be detected in low-abundance using digital high-resolution melt-curve analysis (HRM) combined with pyrosequencing and has been employed successfully to detect mutant DNA in stool[27,28].

Recently, somatic oncogenic mutations in the gene encoding the G protein, Gnas, were identified in 66% of IPMNs[29]. *GNAS* had previously been recognized as an oncogene mutated in the McCune-Albright syndrome[30] and in some pituitary adenomas[31]. Initial evidence indicates that *GNAS* mutations are highly specific for IPMNs[29,32]. Apart from a small percentage (<10%) of PanINs[33], *GNAS* mutations have not been detected in usual pancreatic ductal adenocarcinomas or in mucinous or serous cystic neoplasms[29,32,34].

In this study, we employed digital high-resolution melt-curve analysis and pyrosequencing to measure *GNAS* mutations in secretin-stimulated pancreatic juice and evaluated its diagnostic performance among patients undergoing clinical pancreatic evaluation and subjects undergoing pancreatic screening for their family history of pancreatic cancer.

## MATERIALS AND METHODS

### Patients and specimens

Specimens and clinical information were obtained from 291 participants enrolled in the CAPS clinical trials[8,12]. Subjects enrolled for screening in CAPS trials were asymptomatic individuals with a family history of pancreatic cancer. The CAPS studies also enrolled disease control subjects undergoing evaluation for suspected pancreatic disease primarily to evaluate pancreatic juice markers. CAPS3 subjects were enrolled (2007–2009) at Johns Hopkins Hospital Baltimore, Maryland (JHH), Mayo Clinic (Rochester, Minnesota), Dana Farber Cancer Institute (Boston, Massachusetts), UCLA (Los Angeles, California) and M.D. Anderson Cancer Center (Houston, Texas). For additional disease controls and to include individuals who had undergone serial pancreatic screening evaluations and juice collections, we included JHH subjects enrolled in CAPS2 (2002–2004) and CAPS4 (2008-to-present). Most samples analyzed in this study were from CAPS3 subjects (n=208), although not everyone who participated in CAPS3 provided samples. Fifty samples were from subjects enrolled in CAPS4, 33 from CAPS2 subjects.

Briefly, in CAPS2 and CAPS3, individuals were eligible for screening if they were from familial pancreatic cancer kindreds with three-or-more affected relatives with pancreatic cancer with at least one first-degree relative with familial pancreatic cancer or if they had Peutz-Jeghers syndrome (PJS)[128]. In CAPS4 (ongoing), we enrolled (i) individuals with PJS (ii) individuals typically aged 50 or more with either (a) two-or-more blood relatives with pancreatic cancer and at least one first-degree relative with familial pancreatic cancer or (b) carriers of germline mutations with at least one close blood relative with pancreatic cancer. We referred to these screening risk groups as either (i) “familial” or “strong family history” or (ii) germline mutation carrier subgroups. In addition, in CAPS4 we enrolled subjects for pancreatic surveillance who participated in CAPS2 or CAPS3. Overall, 186 patients were enrolled for their family history of pancreatic cancer alone, two for PJS and 22 with germline mutations (17 *BRCA2*, 3 *BRCA1*, and 2 *p16*). The CAPS studies are described in more detail elsewhere[8,12] (and www.clinicaltrials.gov NCT00438906 and NCT00714701).

The disease controls enrolled included subjects with (i) suspected pancreatic disease who had normal pancreata after pancreatic evaluation (n=20), (ii) chronic pancreatitis diagnosed by pancreatic imaging and clinical criteria (n=20), (iii) IPMN (n=24) diagnosed by either imaging or pathology (table 1), (iv) other pancreatic neoplasms (n=17; 14 pancreatic ductal adenocarcinomas arising in the absence of IPMNs and 3 serous cystadenomas, confirmed by surgical pathology).

After their baseline evaluation asymptomatic subjects undergoing pancreatic screening were classified by imaging as having (i) IPMN(s) (n=54), (ii) diminutive pancreatic cyst(s) (<5mm) (n=33), or (iii) no pancreatic cyst (n=123). Many screened subjects also had subtle architectural changes by EUS resembling chronic pancreatitis possibly from PanIN.[8,15]

Pancreatic juice secretion was stimulated by infusing intravenous human synthetic secretin (0.2 ug/kg over one minute). Pancreatic juice was collected from the duodenal lumen over ~5 minutes as it was secreted by suctioning fluid through the echoendoscope channel. Secretin was provided for CAPS3 and CAPS4 by ChiRhoClin Inc, Burtonsville, Maryland), and for CAPS2 by Repligen Corp (Mass).[12] Juice samples were kept on ice until aliquoting and stored at −80°C prior to use. DNA was extracted from juice samples without additional processing, (we did not separate cells), (using DNeasy Blood & Tissue Kit, QIAGEN) and quantified by Quantifiler (Applied Biosystems).

All elements of this study have been approved by the institutional review boards of all participating sites and written informed consent was obtained from all patients.

**Pancreatic imaging—**Pancreatic imaging for CAPS and criteria used to diagnose IPMNs are described elsewhere[8]. Imaging tests were performed blinded to each other. CT and MRI were performed before radial and linear EUS. After EUS the endosonographer was unblinded to CT/MRI results. The CAPS group held a consensus conference on image interpretation and inter-observer concordance[8]. Interesting cases were reviewed at case conferences. Our CAPS3 study found EUS and MRI/MRCP had excellent concordance (91%), and almost the same detection rate for pancreatic cysts (both superior to CT), with EUS the most sensitive test (further described in[8]). Therefore, when both results were performed, we used EUS results to compare with *GNAS* results. Subjects enrolled in CAPS2 had pancreatic EUS but not MRI/MRCP. Cyst features (location, size, main-duct communication or involvement, septa, mural nodules were noted). For some patients with many cysts (>10), counting may have underestimated the number of cysts. For some cases with many cysts some subcentimeter cysts were not sized, so cyst size/location was described per the largest cyst.

An increase in cyst diameter of ≥3mm between baseline and follow-up exams was defined as an increased in size. Subjects whose cysts increased in size or had new cysts emerge were classified as having cyst progression. Otherwise subjects were defined as having stable cysts. All imaging tests were performed before pancreatic juice analysis.

**High-resolution Digital Melt-curve Analysis—**Ten genome equivalents of juice DNA was dispensed into each well. For each patient's pancreatic juice sample, two 96-well plates of pancreatic juice DNA were analyzed by digital-HRM (180 wells, 1800 genome equivalents, for juice DNA, 10 wells for wild-type DNA, 2 for water). 5μl PCRs were performed with Platinum pfx polymerase (Invitrogen), 0.1 unit/μl LcGreen$^+$ dye (Idaho Tech). *GNAS* primers were 5′-GATTGGCAATTATTACTGTTTC-3′ and 5′-GGAGGAGGACAGCTGGTTATTC-3′. After PCR, plates were cooled to 28°C for 30 seconds to generate heteroduplexes, then subjected to melt-curve analysis (melt-

temperature; 72°C–96°C)(LightScanner mutation analyzer, Idaho Tech). A fluorescence difference of 3% was set as a cutoff for identifying PCR products containing mutant DNA.

**Pyrosequencing—**To confirm digital-HRM results, pyrosequencing was performed on PCR products from HRM-positive, HRM-negative wells and wild-type samples as previously described [30]. A mutation score was generated for each sample (the number of positive wells with *GNAS* codon 201 mutations confirmed by pyrosequencing).

**Laser Captured Microdissection (LCM)—**Available primary neoplastic tissues were analyzed for *GNAS* mutations (8 IPMNs, 10 pancreatic ductal adenocarcinomas (without an associated IPMN) and one serous cystadenoma were microdissected and DNA from neoplastic cells. LCM and mutation analysis was performed as previously described.[30]

**Statistical Analysis—**The prevalence of *GNAS* mutation by disease group was compared by Mann-Whitney. ANOVA was used to compare mutant *GNAS* prevalence in cyst size subgroups. Paired t-test was used to compare baseline vs. follow-up mutant *GNAS* concentrations. Correlations between mutation score, cyst size and number were assessed by scatter-plot and $R^2$ value. Associations between *GNAS* and clinical parameters were evaluated using Fisher's exact test or $\chi^2$ test. Binomial logistic analysis was done including variables with $P<0.05$ as covariates in the final model. Calculations were performed using SPSS Statistics 17.0 (IL, USA). $P<0.05$ was considered statistically significant.

## RESULTS

### Accuracy of pancreatic juice *GNAS* mutations as a test of IPMN

The number of subjects in each diagnostic group is described in Figure 1. Pancreatic juice *GNAS* mutations were detected in 50 of 78 cases with an IPMN, overall sensitivity, 64.1%. In contrast to subjects with IPMNs, no *GNAS* mutations were detected in pancreatic juice samples from the 57 disease control subjects (normal pancreas, chronic pancreatitis and other neoplasms)(Figure 2A). The IPMN group included 54 subjects that had undergone pancreatic screening for their familial risk of pancreatic cancer and 24 individuals with sporadic IPMNs. Two of the surgically-resected IPMNs were main-duct IPMNs. All others were branch-duct IPMNs. There was no difference in mean age between those with IPMNs and disease controls (mean age 57.4/15.2 years). Within the IPMN group, those with "sporadic" IPMNs were older than those with "familial" IPMNs (65.1 vs. 59.2 years, $p=0.009$). Compared to the sporadic IPMNs, pancreatic cysts were smaller (mean/s.d. 6.8mm/4.6 mm) when detected by screening (mean 17.3/9.2mm, $p<0.0001$). Sporadic IPMNs were more likely to be multi-loculated and to have mural nodules (Table 1), consistent with their larger size. There were no significant differences between the sporadic and screening cyst groups with respect to other factors (data not shown).

The spectrum of *GNAS* mutations detected was 34.6%, 21.8% and 5.1% for R201C, R201H and both mutations, respectively. All but one of the patients with two pancreatic juice *GNAS* mutations had multiple cysts. Representative HRM and pyrosequencing results are shown (Figure S1A). There was no significant difference in the prevalence or concentration of mutant *GNAS* detected between pathologically-confirmed IPMNs (n=14) and image-diagnosed IPMNs (n=64) (prevalence; 71.4% and 62.5%, concentration (mean mutation score); 4.3 and 4.6, respectively). As reported previously[29], there was no association between *GNAS* status and neoplastic grade in the resected IPMNs.

The prevalence of juice *GNAS* mutations was not significantly different in those with small vs. larger IPMNs; among IPMNs of 5–9mm (n=40), 10–14mm (n=17) and ≥15mm (n=21),

the prevalence of *GNAS* mutations was 62.5%, 58.8% and 71.4%, respectively, Figure S1B) (Table 1).

The *GNAS* mutations in the resected IPMNs of the 8 patients' whose tissues were available for analysis were uniformly concordant with their corresponding pancreatic juice sample (Figure 3). No *GNAS* mutations were identified in matching neoplastic and pancreatic juice DNA samples from individuals with pancreatic ductal adenocarcinoma (n=10) and serous cystadenoma (n=1).

### Pancreatic juice GNAS mutations in diminutive cystic lesions of the pancreas

We analyzed diminutive pancreatic cysts (defined as cysts <5mm diameter) as a separate group because these cysts are usually indeterminate and are not typically classified as IPMNs.

EUS identified only diminutive cysts (without IPMNs) in 33 of the 156 individuals who underwent screening (Table 2). Pancreatic-protocol CT detected cysts in only 5 of these 33 subjects.

Fifteen (45.5%) of the 33 diminutive cyst subjects had *GNAS* mutations detected in their pancreatic juice (Figure 2B, Figure S1C). Of 123 individuals in the familial pancreatic screening group without a diminutive pancreatic cyst or IPMN, 5 (4.1%) had detectable mutant *GNAS* in their baseline pancreatic juice. The concentration of *GNAS* mutations (mean mutation score) in pancreatic juice was significantly lower in the diminutive cyst group compared to the IPMN group ($P$=0.0063, Figure 2C).

### GNAS status and patient and cyst characteristics

Among individuals diagnosed with sporadic IPMNs, the prevalence of pancreatic juice mutant *GNAS* was 75% which trended higher than the prevalence in the familial screening group (59%, $P$=0.065). Of 111 subjects with pancreatic cysts, univariate analysis found *GNAS* status was associated with; the presence of multiple cysts, septation status, cyst size (<4mm), and patient risk group (sporadic vs. screening group). By multivariate analysis, only cyst number was independently associated with mutant *GNAS* (Table 3). There was no correlation between cyst number and *GNAS* concentration (R=0.03). Among those with pancreatic cysts, *GNAS*-mutant individuals were slightly younger than *GNAS*-wild-type cases (Table 3).

Within the pancreatic screening group, those enrolled for their family history were more likely to have mutant *GNAS* detected than those with known germline mutations (58.4% vs. 10.0%, p=0.0053). This difference was not associated with cyst characteristics. A multivariate analysis of the cyst-positive cases for factors associated with *GNAS* status confirmed familial risk category and cyst multiplicity as independent predictors of *GNAS* mutation status (table S1).

### Serial pancreatic imaging and pancreatic juice analysis

Ninety-seven screened individuals had follow-up pancreatic imaging with either EUS or MRI after their baseline evaluation (median duration, 24 months, range 3–97 months)(table 4, Table S2). Sixty-one of these individuals had serial pancreatic juice collections (table 4); the remaining 36 underwent follow-up pancreatic evaluation without pancreatic fluid collection. Seventy-three individuals had serial EUS and 33 had serial MRI/MRCP. All but 2 cysts found at baseline were detected at follow-up; one small IPMN and one diminutive cyst, both located in the tail of the pancreas, were not seen at follow-up EUS. At follow-up evaluation, cysts emerged in 8 individuals who did not have cysts at baseline, including 3

individuals with normal baseline MRI/MRCP/EUS who had mutant *GNAS* detected in their baseline pancreatic juice (mean follow-up interval; 27.4 months). Two subjects with cysts, including one with diminutive cysts, had mutant *GNAS* only in their second juice sample; the latter subject had new cysts emerge at this follow-up testing. Mutant *GNAS* detected in baseline juice samples was associated with emergence of new cysts at follow-up (mean size, 4.3 mm, $P$=0.0242). One individual with mutant *GNAS* in their pancreatic juice and no pancreatic cysts at either baseline and follow-up exams did have EUS features of chronic pancreatitis suggesting the mutant *GNAS* may have arisen from a lesion too small to be visualized directly[33].

Additionally, 15 subjects had cysts that increased in size or number during follow-up. (One subject's largest cyst increased in size, but had fewer cysts detected). The prevalence of mutant *GNAS* in baseline pancreatic juice samples was significantly higher in subjects whose cysts increased in size compared to those whose pancreatic cysts remained stable ($P$=0.0311). Mutant *GNAS* concentrations did not differ between baseline and follow-up juice samples (data not shown).

## DISCUSSION

Our results indicate that duodenal collections of secretin-stimulated pancreatic juice provide a representative sample of pancreatic secretions that can be used to detect somatic mutations arising in the pancreatic ductal system. We base this conclusion on the high concordance between the detection of mutant *GNAS* in pancreatic juice and the diagnosis of IPMNs. Our prior work found mutant *GNAS* is a specific marker of IPMNs; mutations are present in 66% of resected IPMNs, but not in other cystic neoplasms or pancreatic ductal adenocarcinomas that do not arise in an IPMN[29], and only rarely in PanINs[33]. Combining such a specific marker with sensitive and accurate detection methods (digital-HRM/pyrosequencing) allowed us to evaluate the utility of using secretin-stimulated pancreatic juice as a sample to detect mutations arising in the pancreas. The prevalence of mutant *GNAS* detected in duodenal juice collections of patients with sporadic IPMNs is similar to its prevalence in primary resected IPMNs and IPMN cyst fluids[29]. Our results confirm the very high specificity of mutant *GNAS* for IPMNs; no one in the disease control groups (n=57) had mutant *GNAS* detected in their pancreatic juice.

Another interesting finding is the prevalence of mutant *GNAS* in subjects who had only diminutive cysts, even 1–2mm cysts. We found mutant *GNAS* concentrations were higher in individuals diagnosed with IPMNs than in those with only diminutive cysts. This suggests that mutant DNA concentrations in pancreatic juice collections to some degree reflect the size of the *GNAS*-mutant clone(s) in the pancreas. Furthermore, among subjects with serial measurements, *GNAS* concentrations were almost always consistent. All subjects with mutant *GNAS* detected at baseline had the same *GNAS* mutation detected in their follow-up pancreatic juice sample. One patient with stable-sized cysts between baseline and follow-up pancreatic imaging was mutant *GNAS* detected in their follow-up sample. All other patients who had mutant *GNAS* in only their follow-up juice sample had new cysts emerge at this follow-up evaluation.

Three of the four subjects with mutant *GNAS* in their baseline pancreatic juice but no pancreatic cysts at baseline were later found to have cysts at follow-up. Given the accuracy of our imaging tests, the chance that that imaging missed these baseline cysts is low. This suggests that our mutant *GNAS* measurements were sensitive enough to identify lesions below the limit of detection of (or missed by) pancreatic imaging. The ability of pancreatic juice *GNAS* measurements to herald the subsequent detection of pancreatic cysts adds to existing evidence that *GNAS* mutations can occur in lesions too small to be seen grossly[33],

and that these mutations then presumably drive the growth of these lesions. Overall, our results indicate that secretin-stimulated pancreatic juice samples collected in the duodenum during upper endoscopy are an excellent source of mutant DNA arising from the pancreatic ductal system.

The frequent detection of *GNAS* mutations in subjects with only diminutive cysts indicates that *GNAS* mutations typically arise early in the natural history of an IPMN perhaps even prior to the development of a cyst. Since diminutive cysts have low-malignant potential, the detection of mutant *GNAS* in pancreatic cyst or juice samples should not be an indication to resect the cyst. Criteria for resection should be concern for high-grade dysplasia or invasive cancer (such as the Tanaka criteria[35]).

Current imaging modalities cannot reliably predict the pathology of diminutive pancreatic cysts[36]. Pancreatic cysts of 5–10mm are routinely recognized as IPMNs when they are observed to communicate with the pancreatic duct, but this feature is often not apparent for diminutive cysts. Although IPMNs evolve from smaller lesions, they are not categorized as IPMNs histologically until they are ~1cm[4]. Since diminutive cysts are rarely resected, their pathology is not well understood. Some larger cysts diagnosed by imaging as IPMNs are found at resection to be serous cystadenomas or cystic pancreatic neuroendocrine tumors[343513] Some individuals with small cysts have only PanINs, but not IPMNs, at resection[8,12,37,38], raising the possibility that some diminutive cysts represent PanINs. However, PanINs are not cystic neoplasms. PanINs are routinely identified in pancreata without an identifiable cyst and are much more prevalent than cysts[39]. Instead, it is possible that extensive PanIN could partially obstruct small ductules and the associated small ductule dilation could mimic a cyst. In our series, the prevalence of pancreatic juice *GNAS* mutations in the diminutive cyst cases was not significantly (45.5%) different after multivariate analysis to the prevalence among cases with IPMN(s)(64.1%). This suggests that most diminutive pancreatic cysts are indeed small IPMNs.

We also found preliminary evidence for differences in the prevalence of mutant *GNAS* by screening group. *GNAS* mutations were detected more often in individuals with cysts in the strong family history subgroup than in our small number (n=10) of individuals with known germline mutations (*BRCA2*, *STK11*, *CDKN2A/p16*, and *BRCA1*). We suspect many individuals in the “family history” group carry germline mutations in other pancreatic cancer susceptibility genes that have yet to be identified. The infrequent detection of mutant *GNAS* in some individuals with numerous pancreatic cysts raises the possibility that some individuals develop pancreatic cysts by alternate mechanisms that do not require *GNAS* mutations. Since inherited susceptibility to pancreatic cancer is associated with germline mutations affecting many genes[40], (including *BRCA2*, *p16*, the mismatch repair genes[41], *STK11*, and the more recently identified genes, *PALB2*[42] and *ATM*[43]), it is likely that unique phenotypes and mutational profiles of familial pancreatic neoplasms are yet to be discovered.

The burden of long-term surveillance for most patients with pancreatic cysts necessitates better surveillance strategies. The ability of *GNAS* measurements to herald the development of small pancreatic cysts (that are very likely IPMNs) suggests that pancreatic juice *GNAS* measurements can provide additional information beyond pancreatic imaging about the future risk of developing cystic neoplasms. The detection of mutant *GNAS* provides confirmation that detected pancreatic cysts are neoplastic and if no cysts are detected at the time of the evaluation, our results indicate that pancreatic cysts are likely to be detected in the future. Pancreatic juice analysis could complement FNA analysis which is generally employed to sample larger cysts (>1cm). Ideally, pancreatic juice analysis would include markers that were accurate for identifying low-grade neoplasia such as *GNAS*, and others

accurate for high-grade neoplasia. The absence of accurate markers of low-grade neoplasia would have the potential to predict a very low long-term risk of cancer, analogous to the negative predictive value of a normal screening colonoscopy. More important is to have accurate juice markers for detecting high-grade neoplasia (PanIN-3 and IPMNs with high-grade dysplasia)[44,45]. Since PanINs cannot be reliably visualized with current tests and since most pancreatic ductal adenocarcinomas are thought to develop through the PanIN pathway, pancreatic juice analysis may ultimately provide the best evidence of PanIN short of resecting the pancreas.

Our results demonstrate that digital high-resolution melt-curve analysis combined with pyrosequencing to confirm mutations is an accurate method for detecting mutations present at very low concentrations (0.1% range). Some of the strengths of this study are the large sample size, the multicenter population, including subjects undergoing pancreatic screening and subjects with small and large IPMNs, and the prospective follow-up of some of our study subjects. A limitation of our study was the need to rely on pancreatic imaging to diagnose IPMNs too small to warrant resection. We also classified patient's cyst size by the size of the largest cyst, but other measurements such as the average size of all cysts would be more representative of pancreatic cyst burden.

In conclusion, we find the detection of *GNAS* mutations in duodenal collections of pancreatic juice is a highly specific indicator of pancreatic cysts, and specifically of IPMNs, and the prevalence of mutant *GNAS* in juice samples is similar to that observed in resected IPMNs. We also detect mutant *GNAS* even in juice samples from subjects with the smallest cysts suggesting secretin-stimulated pancreatic juice samples collected from the duodenum are a reliable sample for detecting mutations arising in the pancreatic ductal system. The detection of *GNAS* mutations in pancreatic juice prior to the emergence of a visible IPMN highlights the potential of pancreatic juice analysis to help in the surveillance and risk stratification of patients undergoing pancreatic screening.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Grant Support:** This work was supported by NIH grants (CA62924, R01CA120432, and RC2CA148376), the Lustgarten Foundation for Pancreatic Cancer Research, the Jimmy V Foundation, the Michael Rolfe Foundation, and Karp Family H.H. Metals, Inc. Fund for Cancer Research Michael Hooven and Susan Spies. Material Support: ChiRhoClin (Secretin).

## Abbreviations

| | |
|---|---|
| **IPMN** | intraductal papillary mucinous neoplasm |
| **PanIN** | pancreatic intraepithelial neoplasia |
| **GNAS** | Guanine Nucleotide-Binding Protein, Alpha-Stimulating |
| **EUS** | endoscopic ultrasonography |
| **CT** | computed tomography |
| **MRI/MRCP** | magnetic resonance imaging/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 |

## References


1. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol. 2009; 6:699–708. [PubMed: 19806144]
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62:10–29. [PubMed: 22237781]
3. Salvia R, Fernandez-del Castillo C, Bassi C, et al. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg. 2004; 239:678–85. discussion 85–7. [PubMed: 15082972]
4. Hruban RH, Takaori K, Klimstra DS, et al. An Illustrated Consensus on the Classification of Pancreatic Intraepithelial Neoplasia and Intraductal Papillary Mucinous Neoplasms. Am J Surg Pathol. 2004; 28:977–87. [PubMed: 15252303]
5. Laffan TA, Horton KM, Klein AP, Berlanstein B, Siegelman SS, Kawamoto S, Johnson PT, Fishman EK, Hruban RH. Prevalence of unsuspected pancreatic cysts on MDCT. Am J Radiol. 2008; 191:802–7.
6. de Jong K, Nio CY, Hermans JJ, et al. High Prevalence of Pancreatic Cysts Detected by Screening Magnetic Resonance Imaging Examinations. Clin Gastroenterol Hepatol. 2010; 8:806–11. [PubMed: 20621679]
7. Goggins M. Markers of Pancreatic Cancer: Working Toward Early Detection. Clin Cancer Res. 2011; 17:635–7. [PubMed: 21304000]
8. Canto MI, Hruban RH, Fishman EK, et al. Frequent Detection of Pancreatic Lesions in Asymptomatic High-Risk Individuals. Subtitle: Screening for Early Pancreatic Neoplasia (CAPS 3 Study). Gastroenterology. 2012 in press.
9. Ludwig E, Olson SH, Bayuga S, et al. Feasibility and yield of screening in relatives from familial pancreatic cancer families. Am J Gastroenterol. 2011; 106:946–54. [PubMed: 21468009]
10. Verna EC, Hwang C, Stevens PD, et al. Pancreatic cancer screening in a prospective cohort of high-risk patients: a comprehensive strategy of imaging and genetics. Clin Cancer Res. 2010; 16:5028–37. [PubMed: 20876795]
11. Langer P, Kann PH, Fendrich V, et al. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. Gut. 2009; 58:1410–8. [PubMed: 19470496]
12. Canto MI, Goggins M, Hruban RH, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol. 2006; 4:766–81. [PubMed: 16682259]
13. Canto MI, Goggins M, Yeo CJ, et al. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. Clin Gastroenterol Hepatol. 2004; 2:606–21. [PubMed: 15224285]
14. Brentnall TA, Bronner MP, Byrd DR, et al. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. Ann Intern Med. 1999; 131:247–55. [PubMed: 10454945]
15. Poley JW, Kluijt I, Gouma DJ, et al. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. Am J Gastroenterol. 2009; 104:2175–81. [PubMed: 19491823]

16. Brune K, Goggins M, O'Mailey L, et al. Detailed pathologic evaluation of non-invasive precursor lesions of the pancreas in patients with a strong family history of pancreatic cancer. Am J Surg Pathol. 2006; 30:1067–76. [PubMed: 16931950]

17. Rajan E, Clain JE, Levy MJ, et al. Age-related changes in the pancreas identified by EUS: A prospective evaluation. Gastrointest Endosc. 2005; 61:401–6. [PubMed: 15758911]

18. Matsubayashi H, Canto M, Sato N, et al. DNA methylation alterations in the pancreatic juice of patients with suspected pancreatic disease. Cancer Res. 2006; 66:1208–17. [PubMed: 16424060]

19. Yan L, McFaul C, Howes N, et al. Molecular analysis to detect pancreatic ductal adenocarcinoma in high-risk groups. Gastroenterology. 2005; 128:2124–30. [PubMed: 15940643]

20. Lohr M, Muller P, Mora J, et al. p53 and K-ras mutations in pancreatic juice samples from patients with chronic pancreatitis. Gastrointest Endosc. 2001; 53:734–43. [PubMed: 11375580]

21. Matsubayashi H, Sato N, Brune K, et al. Age- and disease-related methylation of multiple genes in nonneoplastic duodenum and in duodenal juice. Clin Cancer Res. 2005; 11:573–83. [PubMed: 15701843]

22. Ohuchida K, Mizumoto K, Ishikawa N, et al. A highly sensitive and quantitative telomerase activity assay with pancreatic juice is useful for diagnosis of pancreatic carcinoma without problems due to polymerase chain reaction inhibitors: analysis of 100 samples of pancreatic juice from consecutive patients. Cancer. 2004; 101:2309–17. [PubMed: 15476274]

23. Ohuchida K, Mizumoto K, Ogura Y, et al. Quantitative assessment of telomerase activity and human telomerase reverse transcriptase messenger RNA levels in pancreatic juice samples for the diagnosis of pancreatic cancer. Clin Cancer Res. 2005; 11:2285–92. [PubMed: 15788678]

24. Watanabe H, Ha A, Hu YX, et al. K-ras mutations in duodenal aspirate without secretin stimulation for screening of pancreatic and biliary tract carcinoma. Cancer. 1999; 86:1441–8. [PubMed: 10526271]

25. Forsmark CE. The early diagnosis of chronic pancreatitis. Clin Gastroenterol Hepatol. 2008; 6:1291–3. [PubMed: 18986847]

26. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. Am J Gastroenterol. 2002; 97:1386–91. [PubMed: 12094855]

27. Tindall EA, Petersen DC, Woodbridge P, et al. Assessing high-resolution melt curve analysis for accurate detection of gene variants in complex DNA fragments. Hum Mutat. 2009; 30:876–83. [PubMed: 19280649]

28. Zou H, Taylor WR, Harrington JJ, et al. High detection rates of colorectal neoplasia by stool DNA testing with a novel digital melt curve assay. Gastroenterology. 2009; 136:459–70. [PubMed: 19026650]

29. Wu J, Matthaei H, Maitra A, et al. Recurrent GNAS Mutations Define an Unexpected Pathway for Pancreatic Cyst Development. Sci Transl Med. 2011; 3:92ra66.

30. Weinstein LS, Shenker A, Gejman PV, et al. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med. 1991; 325:1688–95. [PubMed: 1944469]

31. Hayward BE, Barlier A, Korbonits M, et al. Imprinting of the G(s)alpha gene GNAS1 in the pathogenesis of acromegaly. J Clin Invest. 2001; 107:R31–6. [PubMed: 11254676]

32. Wu J, Jiao Y, Dal Molin M, et al. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. Proc Natl Acad Sci U S A. 2011; 108:21188–93. [PubMed: 22158988]

33. Kanda M, Matthaei H, Wu J, et al. Presence of Somatic Mutations in Most Early-Stage Pancreatic Intraepithelial Neoplasia. Gastroenterology. 2012; 142:730–3. e9. Epub 2012 Jan 5. [PubMed: 22226782]

34. Furukawa T, Kuboki Y, Tanji E, et al. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep. 2011; 1:161. [PubMed: 22355676]

35. Tanaka M, Chari S, Adsay V, et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology. 2006; 6:17–32. [PubMed: 16327281]

36. Kubo H, Nakamura K, Itaba S, et al. Differential diagnosis of cystic tumors of the pancreas by endoscopic ultrasonography. Endoscopy. 2009; 41:684–9. [PubMed: 19670136]

37. Vasen HF, Wasser M, van Mil A, et al. Magnetic Resonance Imaging Surveillance Detects Early-Stage Pancreatic Cancer in Carriers of a p16-Leiden Mutation. Gastroenterology. 2011; 140:850–6. [PubMed: 21129377]
38. Schneider R, Slater EP, Sina M, et al. German national case collection for familial pancreatic cancer (FaPaCa): ten years experience. Fam Cancer. 2011; 10:323–30. [PubMed: 21207249]
39. Hruban RH, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. Int J Clin Exp Pathol. 2008; 1:306–16. [PubMed: 18787611]
40. Hruban RH, Canto MI, Goggins M, et al. Update on familial pancreatic cancer. Adv Surg. 2010; 44:293–311. [PubMed: 20919528]
41. Kastrinos F, Mukherjee B, Tayob N, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA. 2009; 302:1790–5. [PubMed: 19861671]
42. Jones S, Hruban RH, Kamiyama M, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. Science. 2009; 324:217. Epub 2009 Mar 5. [PubMed: 19264984]
43. Roberts NJ, Jiao Y, Yu J, et al. ATM Mutations in Patients with hereditary Pancreatic cancer. Cancer Discovery. 2011 Published OnlineFirst on December 29, 2011.
44. Yu J, Li A, Hong SM, et al. MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin Cancer Res. 2012; 18:981–92. [PubMed: 22114139]
45. Hong SM, Omura N, Vincent A, et al. Genome-wide CpG island profiling of intraductal papillary mucinous neoplasms of the pancreas. Clin Cancer Res. 2012; 18:700–12. [PubMed: 22173550]

## Significance of this study;

**What is already known about this subject?**

- Pancreatic imaging tests (EUS and/or MRI/MRCP) frequently detect pancreatic cysts, but these tests do not reliably predict cyst pathology. Moreover, many cysts are too small to sample by fine needle aspiration. Better tests are needed to characterize these lesions.
- Secretin-stimulated pancreatic juice samples collected from the duodenum during upper endoscopy is used to diagnose pancreatic insufficiency, but this sample has not been evaluated as a source of mutant DNA from the pancreas.
- *GNAS* mutations are found in ~66% of resected IPMNs but not in other pancreatic cystic neoplasms and so are highly specific for IPMNs,

**What are the new findings?**

- We find mutant *GNAS* in duodenal collections of pancreatic juice from individuals diagnosed with IPMNs just as frequently as in resected IPMNs, and almost exclusively in subjects with pancreatic cysts, including diminutive cysts (<5mm), suggesting that pancreatic juice is a reliable sample for detecting molecular alterations in the pancreatic ductal system.
- We find mutant *GNAS* in some individuals prior to the emergence of a visible pancreatic cyst indicating that *GNAS* mutations arise very early in the natural history of IPMN development.

**How might it impact on clinical practice in the foreseeable future?**

These results highlight the potential of using pancreatic juice analysis with a more comprehensive panel of markers of pancreatic neoplasia to help in the surveillance and risk stratification of patients undergoing pancreatic screening.

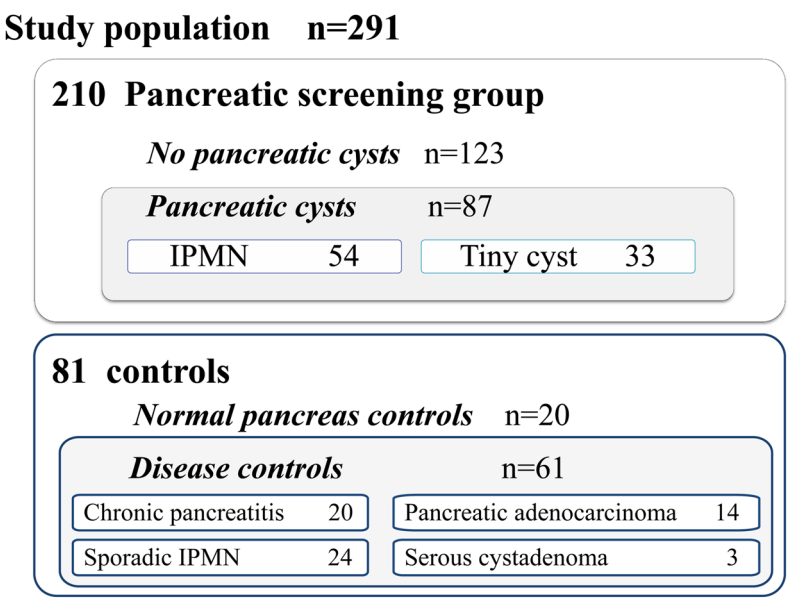


**Figure 1.**
Summary of the study population.

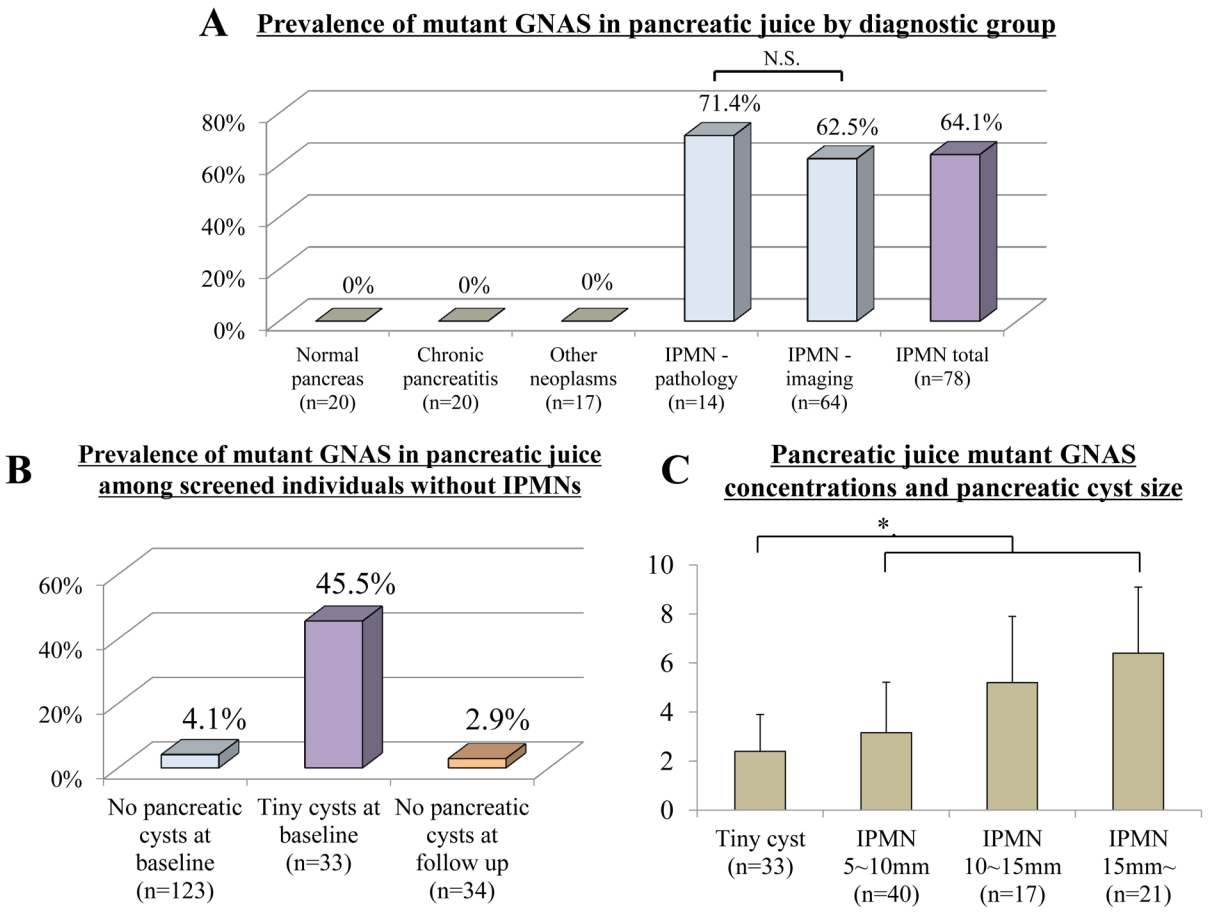


**Figure 2.**

(A) Prevalence of mutant *GNAS* detected in duodenal collections of secretin-stimulated pancreatic juice by patient group. (The IPMN group includes screened and sporadic subjects). (B) Prevalence of pancreatic juice *GNAS* mutations in screened subjects with diminutive cysts or normal pancreata (but not IPMNs). (C) Mutant *GNAS* pancreatic juice concentrations in subjects with pancreatic cyst(s) by cyst size.

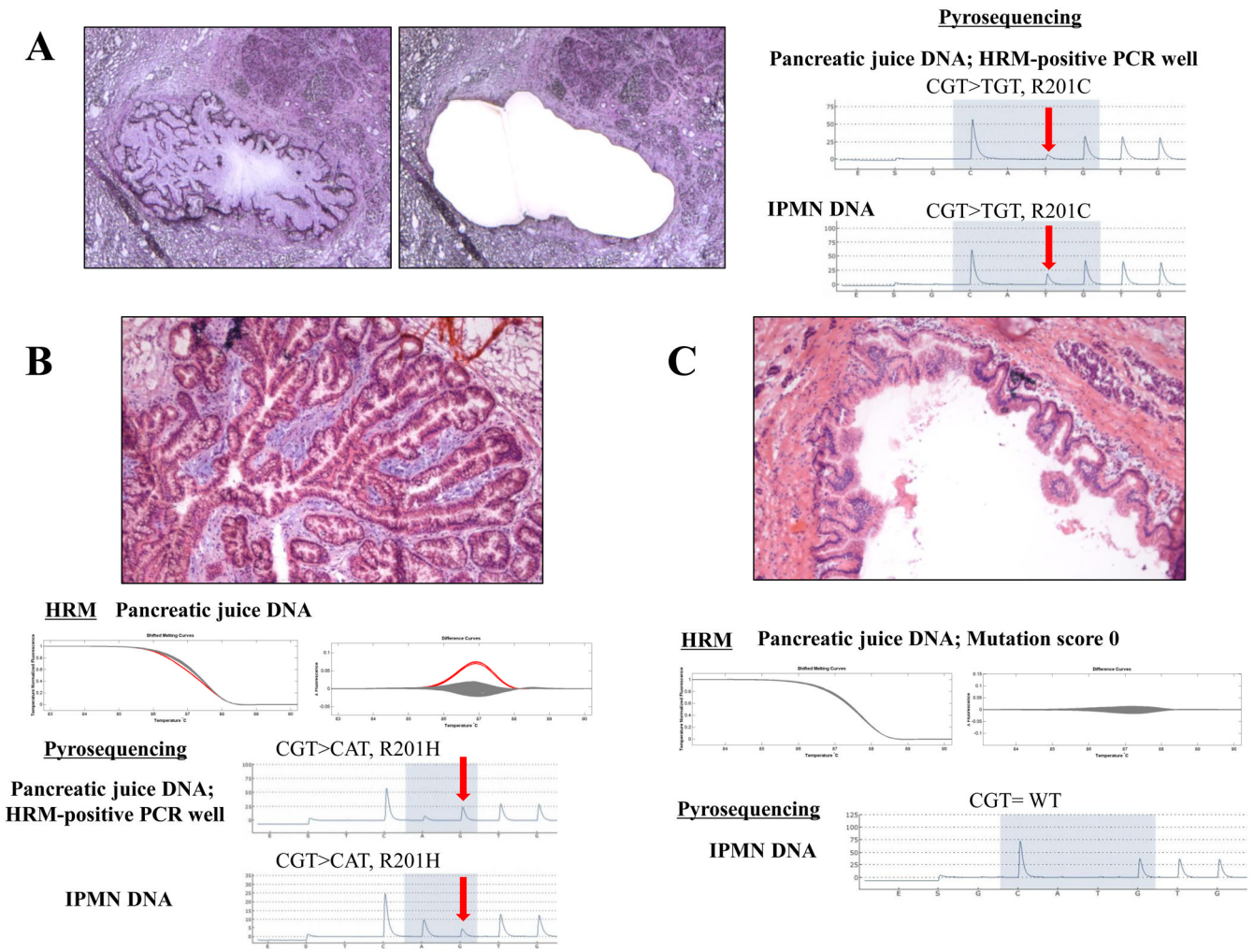


**Figure 3.**

Confirmation of GNAS mutation in corresponding IPMN tissues. (A) An example of an H + E stained (50x) IPMN with moderate-grade dysplasia before and after laser capture microdissection. Microdissection was performed at the duct epithelial cell borders to avoid contamination with stromal cells. GNAS mutation R201C identified in both a HRM-positive well and DNA from the primary IPMN. (B) Microscopic images (100x) of intestine type IPMN with high-grade dysplasia. GNAS mutation R201H identified in both HRM-positive well and DNA from the primary IPMN. (C) Microscopic images (100x) of a gastric-type IPMN with low-grade dysplasia. No GNAS mutations were identified by HRM and wild-type GNAS was confirmed in DNA from the primary IPMN. IPMN, intraductal papillary mucinous neoplasm; HRM, high-resolution melt-curve analysis; WT, wild type. Representative pyrosequencing traces with mutant sequences highlighted by the arrows.

**Table 1**

Clinical features and pancreatic juice mutant *GNAS* status of 78 sporadic and pancreatic screening-identified cases with IPMN(s)

| | Diagnostic test | Risk Group | Gender | Age | Cyst number[a] | Tumor location[b] | Tumor size[b] (mm) | Mural nodule (EUS) | Pathologic grade | GNAS status in juice | | GNAS status in resected IPMN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Mutation score[c] | Mutation | |
| 1 | Pathology | Sporadic | M | 74 | 2 | Head | 50 | 21mm | High | 6 | R201H | |
| 2 | Pathology | Sporadic | M | 49 | 2 | Head | 15 | 5mm | High | 5 | R201C | R201C |
| 3 | Pathology | Sporadic | F | 72 | 2 | Body | 16 | Not detected | Intermediate | 5 | R201C, R201H | R201H |
| 4 | Pathology | Sporadic | F | 66 | 3 | Head | 10 | Not detected | Intermediate | 5 | R201C | R201C |
| 5 | Pathology | Sporadic | M | 64 | 10 | Head | 30 | Not detected | Intermediate | 5 | R201C | |
| 6 | Pathology | Sporadic | F | 66 | 8 | Head | 60 | Not detected | Intermediate | 8 | R201H | |
| 7 | Pathology | Sporadic | F | 66 | 1 | Head | 6 | Not detected | Intermediate | 2 | R201C, R201H | |
| 8 | Pathology | Sporadic | F | 59 | 15 | Tail | 15 | 6mm | Intermediate | 2 | R201C | R201C |
| 9 | Pathology | Familial | F | 72 | 1 | Tail | 6 | Present | Intermediate | 4 | R201C | R201C |
| 10 | Pathology | Sporadic | F | 62 | 3 | Body | 15 | Not detected | Intermediate | 0 | WT | WT |
| 11 | Pathology | PJS | F | 46 | 1 | Uncinate | 10 | Not detected | Intermediate | 0 | WT | WT |
| 12 | Pathology | Sporadic | M | 48 | 1 | Head | 5 | Not detected | Low | 1 | R201C | |
| 13 | Pathology | Familial | M | 56 | 1 | Head | 15 | Not detected | Low | 0 | WT | WT |
| 14 | Pathology | BRCA2[d] | F | 60 | 1 | Tail | 6 | Not detected | Low | 0 | WT | |
| 15 | Imaging | Sporadic | M | 76 | 12 | Body | 18 | Not detected | | 6 | R201C | |
| 16 | Imaging | Sporadic | M | 74 | 1 | Head | 15 | Not detected | | 5 | R201C | |
| 17 | Imaging | Sporadic | F | 86 | 1 | Uncinate | 12 | Not detected | | 4 | R201C | |
| 18 | Imaging | Sporadic | F | 64 | 3 | Body | 12 | Not detected | | 3 | R201C | |
| 19 | Imaging | Sporadic | M | 57 | 3 | Body | 16 | Present 5mm | | 2 | R201C | |
| 20 | Imaging | Sporadic | F | 71 | 2 | Uncinate | 6 | Not detected | | 2 | R201C | |
| 21 | Imaging | Sporadic | M | 60 | 2 | Head | 16 | Not detected | | 5 | R201C, R201H | |
| 22 | Imaging | Sporadic | F | 53 | 2 | Body | 6.4 | Not detected | | 4 | R201C, R201H | |
| 23 | Imaging | Sporadic | M | 75 | 3 | Head | 23 | Not detected | | 7 | R201H | |
| 24 | Imaging | Sporadic | F | 63 | 3 | Body | 19 | Not detected | | 4 | R201H | |
| 25 | Imaging | Familial | M | 67 | 3 | Tail | 9 | Not detected | | 8 | R201C | |
| 26 | Imaging | Familial | F | 59 | 5 | Tail | 12.7 | Not detected | | 7 | R201C | |

| | Diagnostic test | Risk Group | Gender | Age | Cyst number[a] | Tumor location[b] | Tumor size[b] (mm) | Mural nodule (EUS) | Pathologic grade | GNAS status in juice | | GNAS status in resected IPMN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Mutation score[c] | Mutation | |
| 27 | Imaging | Familial | F | 46 | 1 | Head | 9 | Not detected | | 7 | R201C | |
| 28 | Imaging | Familial | F | 64 | 4 | Head | 5.2 | Not detected | | 5 | R201C | |
| 29 | Imaging | Familial | F | 44 | 2 | Tail | 11 | Not detected | | 4 | R201C | |
| 30 | Imaging | Familial | M | 58 | 1 | Body | 5 | Not detected | | 4 | R201C | |
| 31 | Imaging | Familial | M | 66 | 6 | Body | 8.5 | Not detected | | 3 | R201C | |
| 32 | Imaging | Familial | F | 60 | 2 | Head | 8 | Not detected | | 3 | R201C | |
| 33 | Imaging | Familial | F | 58 | 15 | Tail | 8 | Not detected | | 2 | R201C | |
| 34 | Imaging | Familial | F | 52 | 1 | Body | 5.4 | Not detected | | 2 | R201C | |
| 35 | Imaging | Familial | M | 62 | 4 | Tail | 7 | Not detected | | 2 | R201C | |
| 36 | Imaging | Familial | F | 62 | 9 | Tail | 6 | Not detected | | 1 | R201C | |
| 37 | Imaging | Familial | F | 46 | 4 | Body | 8 | Not detected | | 1 | R201C | |
| 38 | Imaging | Familial | F | 59 | 2 | Tail | 9 | Not detected | | 1 | R201C | |
| 39 | Imaging | Familial | M | 61 | 5 | Tail | 7 | Not detected | | 1 | R201C | |
| 40 | Imaging | Familial | M | 67 | 2 | Body | 5 | Not detected | | 6 | R201G | |
| 41 | Imaging | Familial | M | 63 | 15 | Body | 16 | Present | | 13 | R201H | |
| 42 | Imaging | Familial | M | 56 | 2 | Body | 14 | Not detected | | 11 | R201H | |
| 43 | Imaging | Familial | F | 79 | 4 | Tail | 15 | Not detected | | 9 | R201H | |
| 44 | Imaging | Familial | M | 53 | 2 | Body | 12 | Not detected | | 8 | R201H | |
| 45 | Imaging | Familial | M | 63 | 6 | Tail | 18 | Not detected | | 6 | R201H | |
| 46 | Imaging | Familial | M | 70 | 8 | Head | 5 | Not detected | | 6 | R201H | |
| 47 | Imaging | Familial | M | 71 | 2 | Body | 8 | Not detected | | 5 | R201H | |
| 48 | Imaging | Familial | M | 70 | 7 | Tail | 5 | Not detected | | 4 | R201H | |
| 49 | Imaging | Familial | F | 57 | 6 | Body | 8 | Not detected | | 4 | R201H | |
| 50 | Imaging | Familial | M | 54 | 2 | Body | 7 | Not detected | | 4 | R201H | |
| 51 | Imaging | Familial | M | 61 | 2 | Body | 5 | Not detected | | 2 | R201H | |
| 52 | Imaging | Familial | F | 56 | 4 | Body | 5 | Not detected | | 1 | R201H | |
| 53 | Imaging | Familial | F | 46 | 3 | Body | 6.3 | Not detected | | 2 | R201S | |
| 54 | Imaging | Sporadic | M | 59 | 1 | Head | 13 | Not detected | | 0 | WT | |
| 55 | Imaging | Sporadic | F | 75 | 2 | Body | 19 | Present 3mm | | 0 | WT | |

| | Diagnostic test | Risk Group | Gender | Age | Cyst number[a] | Tumor location[b] | Tumor size[b] (mm) | Mural nodule (EUS) | Pathologic grade | GNAS status in juice | | GNAS status in resected IPMN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Mutation score[c] | Mutation | |
| 56 | Imaging | Sporadic | F | 49 | 1 | Tail | 21 | Not detected | | 0 | WT | |
| 57 | Imaging | Sporadic | M | 76 | 1 | Head | 28 | Not detected | | 0 | WT | |
| 58 | Imaging | Sporadic | M | 65 | 4 | Head | 16 | Not detected | | 0 | WT | |
| 59 | Imaging | Familial | F | 55 | 1 | Uncinate | 12 | Not detected | | 0 | WT | |
| 60 | Imaging | Familial | M | 58 | 8 | Head | 11 | Not detected | | 0 | WT | |
| 61 | Imaging | Familial | M | 50 | 10 | Tail | 11 | Not detected | | 0 | WT | |
| 62 | Imaging | Familial | F | 75 | 6 | Tail | 21 | Present | | 0 | WT | |
| 63 | Imaging | Familial | M | 63 | 8 | Tail | 18 | Not detected | | 0 | WT | |
| 64 | Imaging | Familial | M | 63 | 2 | Head | 28 | Not detected | | 0 | WT | |
| 65 | Imaging | Familial | M | 58 | 1 | Head | 5 | Not detected | | 0 | WT | |
| 66 | Imaging | Familial | F | 61 | 4 | Tail | 6 | Not detected | | 0 | WT | |
| 67 | Imaging | Familial | F | 51 | 4 | Tail | 6 | Not detected | | 0 | WT | |
| 68 | Imaging | Familial | M | 74 | 2 | Uncinate | 6 | Not detected | | 0 | WT | |
| 69 | Imaging | Familial | F | 75 | 4 | Tail | 6 | Not detected | | 0 | WT | |
| 70 | Imaging | Familial | F | 50 | 1 | Tail | 6.7 | Not detected | | 0 | WT | |
| 71 | Imaging | Familial | F | 53 | 4 | Body | 5 | Not detected | | 0 | WT | |
| 72 | Imaging | Familial | M | 44 | 1 | Head | 8 | Not detected | | 0 | WT | |
| 73 | Imaging | Familial | M | 57 | 3 | Body | 5 | Not detected | | 0 | WT | |
| 74 | Imaging | Familial | F | 53 | 4 | Body | 6.4 | Not detected | | 0 | WT | |
| 75 | Imaging | Familial | F | 55 | 2 | Tail | 6 | Not detected | | 0 | WT | |
| 76 | Imaging | Familial | M | 61 | 1 | Body | 5.8 | Not detected | | 0 | WT | |
| 77 | Imaging | BRCA1[d] | F | 55 | 3 | Body | 8 | Not detected | | 0 | WT | |
| 78 | Imaging | BRCA2[d] | M | 52 | 8 | Body | 5 | Not detected | | 0 | WT | |

IPMN, intraductal papillary mucinous neoplasm; EUS, endoscopic ultrasonography; HRM, high-resolution melt-curve analysis; WT, wild type; PJS, Peutz-Jeghers syndrome.

[a] cysts seen by EUS.

[b] Tumor location and size of the largest cyst.

[c] Mutation score =total number of positive wells with confirmed GNAS mutations by pyrosequencing.

[d] germline mutation carrier.

**Table 2**

Pancreatic juice *GNAS* status and clinical parameters of 33 screened subjects with only diminutive pancreatic cyst(s)

| | RiskGroup | Gender | Age | EUS findings | | | | | | Detection of cysts by CT | Juice GNAS status | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | # of cysts | Tumor location[a] | Tumor size[a] (mm) | Multi-septal | Duct Communication | Mural nodule | | Mutation score[b] | Codon 201 |
| 1 | Familial | Male | 46 | 1 | Not recorded | 4 | ND | ND | ND | No | 1 | R201C |
| 2 | Familial | Female | 80 | 4 | Tail | 3 | ND | ND | Present | Yes | 1 | R201C |
| 3 | Familial | Female | 50 | 1 | Body | 1 | ND | ND | ND | Yes | 1 | R201C |
| 4 | Familial | Male | 54 | 1 | Body | 4 | ND | ND | ND | No | 3 | R201C |
| 5 | Familial | Female | 59 | 2 | Body | 4 | ND | ND | ND | No | 1 | R201C |
| 6 | Familial | Female | 75 | 4 | Head | 4 | ND | ND | ND | No | 2 | R201C |
| 7 | Familial | Male | 62 | 1 | Body | 4 | ND | Present | ND | No | 2 | R201C |
| 8 | Familial | Male | 67 | 1 | Body | 4 | ND | ND | ND | No | 2 | R201C |
| 9 | Familial | Female | 64 | 2 | Tail | 4 | ND | ND | Present | Yes | 3 | R201H |
| 10 | Familial | Male | 66 | 2 | Head | 3 | Present | ND | ND | No | 3 | R201H |
| 11 | Familial | Male | 73 | 1 | Tail | 4 | ND | ND | ND | No | 3 | R201H |
| 12 | Familial | Male | 56 | 4 | Head | 3 | ND | ND | ND | No | 5 | R201H |
| 13 | Familial | Female | 60 | 3 | Head | 3 | ND | ND | ND | No | 2 | R201H |
| 14 | Familial | Female | 59 | 2 | Head | 4 | ND | ND | ND | No | 6 | R201H |
| 15 | BRCA2[c] | Male | 62 | 2 | Body | 4 | ND | ND | ND | No | 1 | R201C |
| 16 | Familial | Male | 43 | 5 | Not recorded | 4 | ND | ND | ND | No | 0 | WT |
| 17 | Familial | Female | 54 | 6 | Body | 4 | ND | ND | ND | Yes | 0 | WT |
| 18 | Familial | Female | 48 | 4 | Tail | 3 | ND | ND | ND | Yes | 0 | WT |
| 19 | Familial | Male | 54 | 2 | Body | 2 | ND | ND | ND | No | 0 | WT |
| 20 | Familial | Male | 61 | 1 | Body | 2 | ND | ND | ND | No | 0 | WT |
| 21 | Familial | Male | 50 | 1 | Body | 2 | ND | ND | ND | No | 0 | WT |
| 22 | Familial | Female | 55 | 1 | Head | 3 | ND | ND | ND | No | 0 | WT |
| 23 | Familial | Male | 63 | 1 | Body | 3 | ND | ND | ND | No | 0 | WT |
| 24 | Familial | Male | 70 | 1 | Head | 4 | ND | Present | ND | No | 0 | WT |
| 25 | Familial | Female | 54 | 1 | Body | 4 | Present | ND | ND | No | 0 | WT |
| 26 | Familial | Male | 61 | 2 | Head | 3 | ND | ND | ND | No | 0 | WT |
| 27 | Familial | Male | 60 | 1 | Body | 2 | ND | Present | ND | No | 0 | WT |

| | RiskGroup | Gender | Age | EUS findings | | | | | | Detection of cysts by CT | Juice GNAS status | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | # of cysts | Tumor location[a] | Tumor size[a] (mm) | Multi-septal | Duct Communication | Mural nodule | | Mutation score[b] | Codon 201 |
| 28 | Familial | Male | 71 | 1 | Body | 4 | ND | ND | ND | No | 0 | WT |
| 29 | BRCA2[c] | Female | 59 | 1 | Tail | 2 | ND | ND | ND | No | 0 | WT |
| 30 | BRCA2[c] | Female | 72 | 1 | Body | 2 | ND | Present | ND | No | 0 | WT |
| 31 | BRCA2[c] | Male | 49 | 2 | Tail | 2 | ND | Present | ND | No | 0 | WT |
| 32 | p16[c] | Male | 70 | 1 | Body | 4 | ND | ND | ND | No | 0 | WT |
| 33 | PJS | Female | 32 | 1 | Tail | 2 | Present | ND | ND | No | 0 | WT |

EUS endoscopic ultrasonography; WT, wild type; PJS, Peutz-Jeghers syndrome.

[a] size of the largest cyst.

[b] Mutation score was calculated from total number of positive wells with confirmed GNAS mutations by pyrosequencing. ND, Not detected.

[c] Carriers of germline mutation.

**Table 3**

Associations between pancreatic juice *GNAS* and clinical parameters in 111 subjects with pancreatic cysts

| | GNAS mutation (n) | GNAS WT (n) | Univariate | Multivariate | | |
|---|---|---|---|---|---|---|
| | | | ***P* value** | **HR** | **95%CI** | ***P* value** |
| Age (mean/sd, years) | 58.4/9.6 | 62.0/9.2 | **0.0492** | | | |
| Gender | | | | | | |
| Male | 32 | 25 | 0.5952 | | | |
| Female | 33 | 21 | | | | |
| Background | | | | | | |
| Sporadic | 19 | 5 | **0.0206** | 2.616 | 0.837 – 8.171 | 0.0980 |
| High risk individuals | 46 | 41 | | | | |
| High Risk subgroup[a] | | | | | | |
| Familial | 45 | 32 | **0.0053** | | Not applicable | |
| Familial + Germline mutation[b] | 1 | 9 | | | | |
| Cyst number | | | | | | |
| Solitary | 15 | 22 | **0.0064** | 2.584 | 1.063 – 6.289 | **0.0362** |
| Multiple | 50 | 24 | | | | |
| Tumor Size | | | | | | |
| ≤ 3mm | 5 | 12 | **0.0080** | 2.585 | 0.770 – 8.685 | 0.1244 |
| > 3mm | 60 | 34 | | | | |
| Cyst Location | | | | | | |
| Head and uncinate | 22 | 16 | 0.9184 | | | |
| Body and tail | 43 | 30 | | | | |
| Multi-septal formation | | | | | | |
| Absent | 28 | 30 | **0.0214** | 1.375 | 0.567 – 3.336 | 0.4807 |
| Present | 37 | 16 | | | | |
| Duct communication | | | | | | |
| Absent | 49 | 32 | 0.4964 | | | |
| Present | 16 | 14 | | | | |
| Mural nodule | | | | | | |
| Absent | 57 | 44 | 0.1907 | | | |

| | GNAS mutation (n) | GNAS WT (n) | Univariate | | Multivariate | |
|---|---|---|---|---|---|---|
| | | | *P* value | HR | 95%CI | *P* value |
| Present | 8 | 2 | | | | |
| Dilation of the branch ducts | | | | | | |
| Absent | 54 | 34 | 0.2406 | | | |
| Present | 11 | 12 | | | | |
| Dilation of the main pancreatic duct | | | | | | |
| Absent | 51 | 38 | 0.5892 | | | |
| Present | 14 | 8 | | | | |
| Chronic pancreatitis | | | | | | |
| Absent | 48 | 34 | 0.9937 | | | |
| Present | 17 | 12 | | | | |

IPMN, intraductal papillary mucinous neoplasm; WT, wild type; HR, hazard ratio.

[a] Sporadic patients excluded.

[b] BRCA2, BRCA1, p16, STK11.