



Pancreatic cyst fluid harboring a *KRAS* mutation

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Abstract A 55-yr-old woman presented with abdominal bloating for approximately 1 year. Imaging studies showed a cyst in the body of the pancreas with proximal pancreatic ductal dilation. An endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was performed. Cytologic findings from the cyst fluid were consistent with a mucinous neoplastic cyst, and the possibility of malignancy could not be entirely excluded. A *KRAS* mutation analysis was performed on the cyst fluid using the Idylla system and circulating tumor *KRAS* (ct*KRAS*) cartridge (Biocartis, Mechelen, Belgium), which tests for actionable mutations in exons 2, 3, and 4 of the *KRAS* gene. Idylla testing detected a *KRAS* G12D mutation in the cyst fluid. The patient subsequently underwent a distal subtotal pancreatectomy with splenectomy. Microscopic examination of the resected tissue revealed an intraductal papillary mucinous neoplasm (IPMN) with an associated invasive carcinoma. *KRAS* testing on the resected tumor tissue confirmed the G12D mutation detected in the cyst fluid earlier. The described rapid testing of *KRAS* directly from the pancreatic cyst fluid can complement cytology assessment to classify pancreatic cysts more reliably and can potentially be of significant help when other cyst findings are nondiagnostic.

[Supplemental material is available for this article.]

CASE PRESENTATION

The patient is a 55-yr-old woman who has a past medical history that included a right mastectomy with contralateral prophylactic mastectomy (bilateral) in 2010. She was found to have a *BRCA2* mutation, Y3308X, which she inherited from the paternal side of her family. This Y3308X mutation results in premature termination of the *BRCA2* protein at amino acid 3308. Her father was diagnosed with a *BRCA2* gene mutation and her paternal grandfather died of pancreatic cancer. She has several paternal aunts with breast cancer. On the maternal side of the family, her grandmother also died of pancreatic cancer.

The patient self-reported feeling bloated for approximately 1 year and a CT scan was ordered, which showed a cyst in the body of the pancreas with proximal pancreatic ductal dilation. Because of her *BRCA2* status and the concern for pancreatic cancer (Mersch et al. 2015), an endoscopic ultrasound (EUS) was performed. An anechoic lesion suggestive of a cyst was identified in the pancreatic body and it communicated with the pancreatic duct that was moderately dilated upstream (3.5 mm) (Fig. 1A). The lesion measured 29 mm by 22 mm in maximal cross-sectional diameter. There were a few compartments thickly septated. The outer wall of the lesion was thin. There was no associated mass. There was no internal debris within the fluid-filled cavity. An endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was performed and the fluid was clear and watery. Samples were sent for amylase

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Ontology term: pancreatic cysts

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a003830

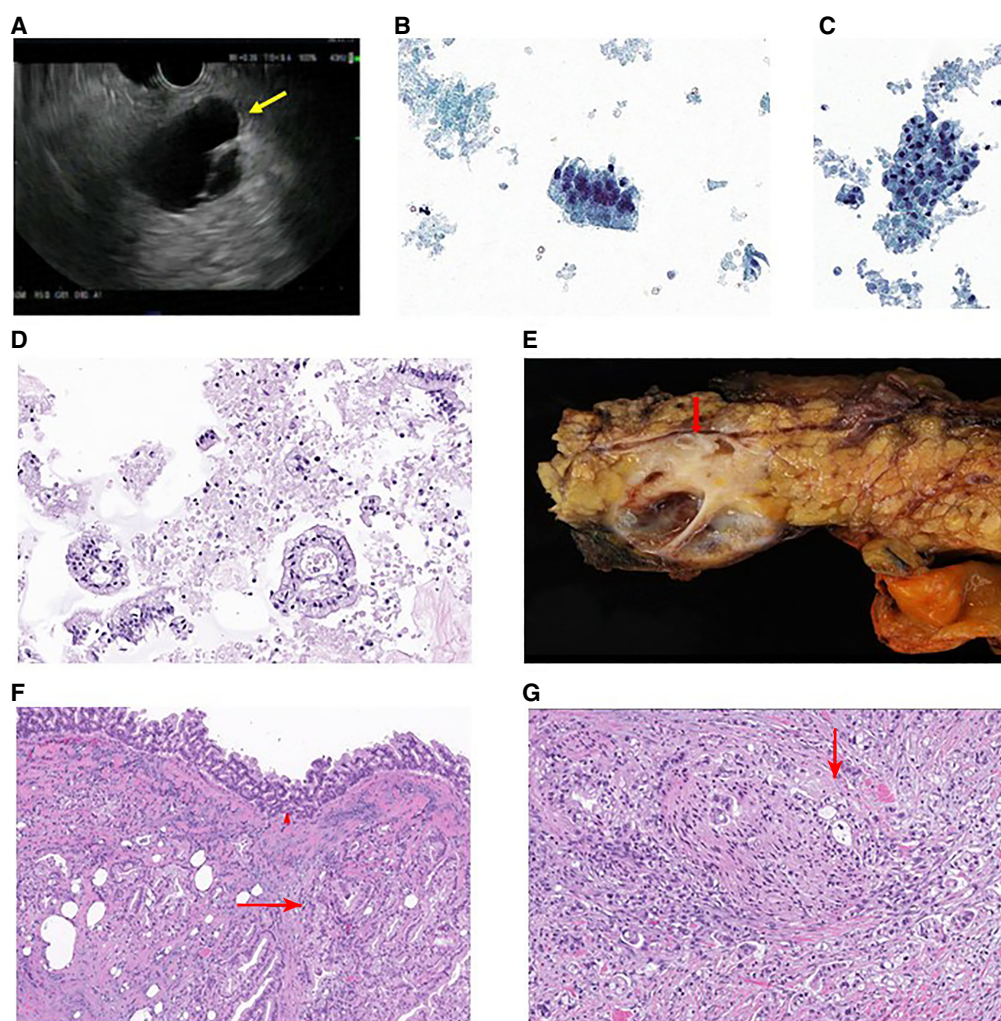


Figure 1. (A) Endoscopic ultrasound (EUS) showing a 29-mm septated cystic lesion seen in the pancreatic body, associated with upstream ductal dilation. The outer wall of the lesion was thin. There was no associated mass. (B) Mucinous/gastric foveolar-type epithelial cells in a small cluster. (C) Focal cytologic and architecture atypia and background necrosis. (D) Cell block showing a few clusters of mucinous/gastric foveolar-type epithelial cells and background necrosis. (E) Cyst at the body of pancreas measuring 4.1 × 2.2 × 2.0 cm with solid area extending into the pancreatic duct (arrow). (F) IPMN (arrowhead) with an associated invasive carcinoma (arrow). (G) Invasive carcinoma with perineural invasion (arrow).

and carcinoembryonic antigen (CEA) testing as well as cytopathologic examination. There was no sign of significant endosonographic abnormality in the common bile duct. There was an 8-mm stone in the gallbladder. No lymphadenopathy was seen.

On cytologic examination, single and clusters of mucinous/gastric foveolar-type epithelial cells were present (Fig. 1B). This was consistent with a mucinous neoplastic cyst. Focal cytologic atypia with possible necrosis was seen, and the possibility of malignancy could not be entirely excluded (Fig. 1C). The cyst fluid amylase was 66 units/L and the CEA was 8334 ng/mL.

The patient denied any nausea, anorexia symptoms, or early satiety. She initially had no weight loss but later started showing dyspepsia-like symptoms and lost nearly 10 pounds. There were no symptoms of pancreatic exocrine insufficiency or pancreatitis. Based on the

cyst size, its relatively thick septations, and atypical features on cytology, she was referred to surgery for resection.

A distal subtotal pancreatectomy with splenectomy was performed, which showed a 4.1 × 2.2 × 2 cm cyst in the body of the pancreas. The cyst had a solid area extending into the pancreatic duct (Fig. 1E). Microscopic examination revealed an intraductal papillary mucinous neoplasm (IPMN) with an associated invasive carcinoma (Fig. 1F). Perineural invasion was present (Fig. 1G). The tumor invaded peripancreatic soft tissues, and all margins were uninvolved by invasive carcinoma. All examined lymph nodes were negative for carcinoma.

TECHNICAL ANALYSIS AND METHODS

A 100- μ L aliquot of the pancreatic cyst fluid was analyzed shortly after EUS-FNA using the Idylla system (Biocartis, Mechelen, Belgium), which is a cartridge-based molecular system that provides automated sample processing and targeted mutation detection directly from formalin-fixed, paraffin-embedded (FFPE) tissue or plasma samples, with all reagents included in a single-use cartridge. The specific cartridge assay used for molecular analysis of the cyst fluid was the ctKRAS assay, which is designed to detect *KRAS* mutations in circulating tumor DNA using 1 mL of plasma directly added to the cartridges. In our case, we directly added cyst fluid to the cartridge. The cartridge tests for 21 mutations in exons 2, 3, and 4 of the *KRAS* gene and the analytical sensitivity is $\leq 1\%$ for mutations in exons 2 and 3 and $\leq 5\%$ for mutations in exon 4 (Supplemental Table S1). During testing, DNA liberation, PCR amplification, and detection occur in the cartridge for a total run time of ~ 2 h. Additional *KRAS* testing on the resected tumor tissue was performed 3 mo after cyst fluid testing using one 10- μ m FFPE tissue section and the Idylla *KRAS* cartridge, which tests tissue sections for the same *KRAS* mutations as the ctKRAS cartridge.

The Idylla assays use a real-time PCR chemistry based on PlexPrime and PlexZyme (also known as MNazyme) technology (Mokany et al. 2010). With this technology, each primer is designed to have a 5' target-recognition region, a short 3' target-specific sequence complementary to the mutation of interest, and a distinct insert sequence that is mismatched to the target. This results in the production of allele-specific amplicons that are detected in real time by allele-specific PlexZyme enzymes and a universal fluorescent probe, allowing for detection of multiple mutations in a single multiplex reaction. Several studies have been performed on the Idylla system using cancer specimens from different tissues and they showed excellent concordance with other available molecular methods, including next-generation sequencing (Al-Turkmani et al. 2018; Uguen and Tronccone 2018).

VARIANT INTERPRETATION

The Idylla testing detected a *KRAS* G12D (c.35G>A) mutation in the pancreatic cyst fluid. This single-nucleotide mutation in codon 12 of exon 2 results in replacement of the GGT codon for glycine with the GAT codon for aspartic acid, leading to impairment of the intrinsic GTPase activity of RAS and subsequent activation of downstream signaling pathways that drive cancer growth (Table 1). Subsequent *KRAS* testing of the resected tumor tissue confirmed the G12D mutation detected earlier in the pancreatic cyst fluid.

Table 1. Variant table

Gene	Chromosome	HGVS DNA	HGVS protein	Variant type	Predicted effect
<i>KRAS</i>	12	c.35G>A	p.G12D	Substitution	Impairment of GTPase activity

Pancreatic cancer is the fourth leading cause of cancer death in the United States, and it is expected to be the second by 2030 (Rahib et al. 2014). The activating point mutation of the *KRAS* oncogene in codon 12 is present in up to 94% of pancreatic ductal adenocarcinoma (PDAC) and it appears to be the initiating genetic event for this malignancy (Waters and Der 2018). PDAC patients harboring the *KRAS* G12D mutation, the same mutation detected in the pancreatic cyst fluid and tumor tissue here, were found to have a significantly shorter overall survival compared with other patients with wild-type *KRAS* or other codon 12 *KRAS* mutations (Bournet et al. 2016).

Many of the pancreatic cystic neoplasms, including serous cystadenomas, are benign and can be monitored periodically. In contrast, IPMNs and mucinous cystic neoplasms (MCNs) have the potential to progress to invasive pancreatic adenocarcinoma. Recently, DNA-based testing has emerged as an adjunct test to the assessment of pancreatic cysts. In a prospective, multicenter study that included 113 patients with 40 malignant, 48 pre-malignant, and 25 benign pancreatic cysts, *KRAS* mutation was helpful in identifying mucinous cysts with 96% specificity (Khalid et al. 2009). Another more recent study on 52 pancreatic cyst fluids classified as mucinous (40%) or nonmucinous (60%) found that cysts harboring *KRAS* mutations were more commonly mucinous and malignant (Faias et al. 2018). An additional study on 626 pancreatic cyst fluid samples that were tested preoperatively by NGS-identified *KRAS*/*GNAS* mutations in 49% of the samples, and these mutations were highly sensitive for IPMNs and specific for mucinous pancreatic cysts (Singhi et al. 2018). Combining *KRAS*/*GNAS* mutations to alteration in *TP53*/*PIK3CA*/*PTEN*, present in 6% of cases, was a useful preoperative marker for advanced neoplasia. Based on these studies, detection of *KRAS* mutations can complement cytology assessment in order to more reliably detect malignant mucinous lesions.

SUMMARY

Direct, rapid mutation analysis of *KRAS* in pancreatic cyst fluid was achieved in the presented case using the Idylla ct*KRAS* Mutation Assay. The detected *KRAS* G12D mutation further supported the mucinous nature of the cyst. Such *KRAS* analysis can be of significant help in identifying mucinous cysts when cyst morphology and cytology are nondiagnostic. The high sensitivity of the Idylla assay and the minimal amount of sample required for testing make it particularly helpful for cases where cellular content and fluid volume of pancreatic cysts are suboptimal for other routine ancillary studies, such as cytopathologic examination and CEA quantitation.

Unlike most molecular methods currently available for somatic mutation testing, Idylla testing can be performed directly on body fluids, plasma, or FFPE tissue sections without the need for sample preprocessing or extraction. The system also allows for random access testing, which results in rapid turnaround times. Additionally, Idylla requirements for space and infrastructure are minimal, allowing for its placement outside the central laboratory for expedited testing process and timely patient management decisions. A limitation of the Idylla testing is that it does not provide mutation allele fraction (MAF), which would provide further insight into the neoplastic cellularity of the tumor. Another limitation is the limited number of *KRAS* mutations the ct*KRAS* cartridge tests for and the lack of assays that test for mutations in other genes that play a key role in the development and progression of pancreatic cancer including *CDKN2A*, *SMAD4*, and *TP53*.

To our knowledge, this is the first United States–based report describing the Idylla ct*KRAS* testing and its use with pancreatic cyst fluids. Further studies comparing pancreatic cyst fluid results by the Idylla to those obtained from concurrent testing of the cell block or resected tumor tissue would help establish the analytical sensitivity and specificity of the

Idylla ctKRAS assay. Our study offers a proof of concept and promise of providing simple and fast KRAS mutation analysis in pancreatic cyst fluids.

ADDITIONAL INFORMATION

Data Deposition and Access

The described variant has been submitted to ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>) and can be found under accession number SVC000882700.

Ethics Statement

The study was approved by the Committee for the Protection of Human Subjects at Dartmouth-Hitchcock Medical Center, and the results obtained were not used for diagnostic purposes of any kind.

Acknowledgments

We thank the staff of the Laboratory for Clinical Genomics and Advanced Technology (CGAT) at Dartmouth-Hitchcock Medical Center.

Author Contributions

All authors contributed to scientific discussion and manuscript review.

Funding

This work was funded by the Department of Pathology at Dartmouth-Hitchcock Medical Center.

Competing Interest Statement

The authors have declared no competing interest.

Received November 30, 2018;
 accepted in revised form
 February 1, 2019.

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