

Patient
DOB/Age/Sex
Client Identifier
Collection Date
Accession Date
Reported Date

Accession #: **MGP22-**
Client Accession #:
Client / Ordering Laboratory
Requesting Physician
Ordering Physician

CLINICAL HISTORY

Specimen Type: Pancreas, Cyst fluid Location: Pancreatic Tail Cyst

PANCREASEQ® GC RESULTS SUMMARY

Test Result	Type of Cyst	Risk of High-Grade Dysplasia/Cancer
POSITIVE	Likely Neuroendocrine (NET)	Likely Low <i>*See interpretation below for details</i>

INTERPRETATION

- MEN1 mutation together with high expression of the neuroendocrine marker, Chromogranin A were identified in this sample.
- The detection of MEN1 alterations and the absence of KRAS and GNAS mutations are associated with a cystic pancreatic neuroendocrine tumor (PanNET).
- Copy number alterations or other high risk molecular alterations were not detected.
- Patient management decisions must be based on the independent medical judgment of the treating physician. Molecular test results should be taken into consideration in conjunction with all relevant imaging and clinical findings, patient and family history, as well as patient preference.

DETAILED RESULTS

Sample cellularity: **ADEQUATE**

Marker Type	Marker Result	AF	Class
Gene mutations	MEN1 p.T150Pfs*35 c.448del	38%	Tier 1/2
Gene fusions	Negative		
Copy number alterations	Negative		
Neuroendocrine markers	Positive		
CEACAM5 (CEA) RNA expression	0 GEU		

AF=Variant Allele Frequency, Tier 1/2=Variants of Clinical or Potential Clinical Significance, VUS=Variants of Uncertain Significance
GEU=Gene Expression Units

BACKGROUND

Both pancreatic cysts and pancreatic solid lesions represent a broad and diverse group of benign and malignant entities. Among pancreatic cysts, distinguishing one pancreatic cyst from another can be challenging on the basis of standard clinical findings, imaging parameters and ancillary fluid studies, such as cytology and CEA analysis. DNA and RNA sequencing studies of pancreatic cysts have identified a number of genetic alterations that can be used diagnostically and prognostically to classify pancreatic cysts.(1-7,12) Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) represent mucinous pancreatic cystic neoplasms. Over 95% of IPMNs are characterized by mutations in the genes: KRAS (codons 12, 13 and/or 61), GNAS (codons 201 and 227), RNF43, BRAF, and CTNNB1.(1-4, 7-10) KRAS, RNF43 and CTNNB1 mutations can also be found in MCNs with a prevalence that ranges from 14% to 50%.(1-4, 7-10) In contrast to IPMNs, MCNs do not harbor GNAS and BRAF mutations, and, thus, genetic alterations in GNAS and BRAF are highly specific for IPMNs.(2-4, 7-10) Other neoplastic cysts include serous cystadenomas and solid pseudopapillary neoplasms. Serous cystadenomas (SCAs) have an extremely low malignant potential and approximately 89% to 100% harbor mutations and/or deletions in VHL, but lack mutations in KRAS, GNAS and BRAF.(3, 4, 7-10) Finally, solid-pseudopapillary neoplasms (SPNs) are characterized by the presence of CTNNB1 mutations (within exon 3), and an absence of alterations in KRAS, GNAS, RNF43, BRAF and VHL.(2-4, 7-10)

IPMNs and MCNs are precursor neoplasms to pancreatic ductal adenocarcinoma; however, only a subset harbor or progress to malignancy. Studies have shown that IPMNs and MCNs with genetic alterations in TP53, SMAD4 and the phosphatidylinositol-3 kinase (PI3K) pathway, which include PIK3CA, PTEN, and AKT1, are associated with high-grade dysplasia and early invasive pancreatic ductal adenocarcinoma (PDAC). (2, 3, 7, 8, 11, 12) Kameta et al demonstrated that NGS for KRAS, TP53 and SMAD4 alterations on EUS-FNA specimens is associated with a 96%, 44% and 11% sensitivity, respectively, and 100% specificity for PDAC.(14) Similarly, Young and colleagues found EUS-FNA specimens harboring mutations in KRAS, TP53 and/or SMAD4 were present in 95% of cases that correlated with PDAC.(15) Within a large cohort of EUS-FNA specimens, Gleeson et al. found KRAS, TP53 and SMAD4 alterations were present in 93%, 72% and 31% of PDACs.(16)

Cystic pancreatic neuroendocrine tumors (PanNETs) are typically diagnosed by standard cytology, but the diagnosis may be facilitated by the detection of specific molecular alterations. PanNETs do not have KRAS mutations, but harbor frequent alterations in MEN1, VHL, and/or TSC2.(2-4, 17) Further, recurrent genomic alterations in several chromatin remodeling genes leads to numerous chromosomal copy number alterations, which is associated with decreased disease-free survival and decreased disease-specific survival.(2,3) This is especially critical when evaluating small neuroendocrine tumors.(17) Genetic alterations are absent in benign non-neoplastic cysts, such as pseudocysts, lymphoepithelial cysts, retention cysts, squamous cysts or acinar cell cystadenomas.(2,3)

Utility of molecular markers have been discussed by the International Consensus Fukuoka Guidelines for the management of IPMNs and MCNs, and the European Evidence-Based Guidelines on pancreatic cystic neoplasms where their role in the diagnosis of pancreatic cysts was highlighted.(7, 8, 13)

A pancreatic cyst fluid carcinoembryonic antigen (CEA) is a useful marker in identifying mucinous cysts. The CEACAM5 gene encodes a cell surface glycoprotein that plays a role in cell adhesion, intracellular signaling and tumor progression and is the founding member of the carcinoembryonic antigen (CEA) family of proteins. Measuring mRNA expression of the CEACAM5 gene in pancreatic cyst fluid samples can be used to detect CEA upregulation. (18)

References

1. Singhi AD, et al. Gut. 2018;;
2. Panizza A, et al. Gastroenterology. 2022;;
3. Nikiforova MN, et al. Ann Surg. 2023;;
4. Springer S, et al. Sci Transl Med. 2019;11:eaav4772;
5. Singhi AD, et al. Clin Cancer Res. 2014;20:4381-9;
6. Nikiforova MN, et al. Mod Pathol. 2013;26:1478-87;
7. Bell PD, et al. Surg Pathol Clin. 2022;15:455-468;
8. Pitman MB, et al. Acta Cytol. 2023;67:304-320;
9. Singhi AD, et al. Gastroenterology. 2020;158:573-582.e2;
10. Amato E, et al. J Pathol. 2014;233:217-27;
11. Singhi AD, et al. Nat Rev Gastroenterol Hepatol. 2021;18:457-468;
12. Singhi AD, et al. Gastrointest Endosc. 2016;83:1107-1117.e2;
13. Tanaka M, et al. Pancreatol. 2017;17:738-753;
14. Kameta E, et al. Oncol Lett. 2016;12:3875-3881;
15. Young G, et al. Cancer Cytopathol. 2013;121:688-94;
16. Gleeson FC, et al. Oncotarget. 2016;7:54526-54536;
17. Pea A, et al. Ann Surg. 2018;;
18. Vuijk FA, et al. Sci Rep. 2020;10:16211;

METHODOLOGY

Nucleic acids are isolated from pancreatic cyst fluid samples collected in the FNA Preserve solution using standard laboratory procedures. The NGS analysis is performed to detect SNVs and indels in the AKT1, APC, BRAF, CTNNB1, GNAS, HRAS, IDH1, IDH2, KRAS, MEN1, MET, NF2, NRAS, PIK3CA, PTEN, STK11, TERT, TP53, TSC2, and VHL genes; copy number alterations (CNAs) at 13 chromosomal regions, including loss of heterozygosity (LOH) in the RNF43 (17q), SMAD4 (18q), TP53 (17p), VHL (3p), NF2 (22q), PTEN (10q) tumor suppressor genes; gene fusions in the ALK (GFPT1, GTF2IRD1, EML4, TFG, CCDC149, STRN), BRAF (AGK, BCL2L11, TRIM24, POR, SND1, AKAP9, ZC3HAV1, MKRN1, PICALM, CCNY, GORASP2, AGK, FAM114A2, MACF1, ZBTB8A), ERBB4 (EZR), NTRK1 (TPM3, TFG, TPR, SSBP2, SQSTM1, IRF2BP2, BANP, ETV6), NTRK3 (SQSTM1, EML4, ETV6, RBPMS), ROS1 (CCDC30), RAF1 (AGGF1), PRKACB (ATP1B1, DNAJB1), and PRKACA (ATP1B1); and gene expression of CEACAM5, GUS, KRT7, KRT20, CHRGR, PGK1.

Clinical validity and utility of the PancreaSeq test was established in several studies including a large prospective multicenter study of 1,889 patients with pancreatic cysts reported in Gastroenterology (1,2). In addition, an updated PancreaSeq Genomic Classifier (GC) DNA/RNA next-generation sequencing test was validated in a large multicenter study and reported in Annals of Surgery (3). Analytical sensitivity (PPA) and analytical specificity (PPV) for SNVs/indels is >99%/99% at 3-5% AF (6-10% of neoplastic cells), for GF is >99%/99% at >1-3% neoplastic cells, for GEA is >99%/99% at 10% neoplastic cells, and for CNA is 92%/100% with LOD 20-25% of neoplastic cells. The assay minimal required sequencing depth is 500x. Genetic regions that did not meet minimal sequencing coverage requirements are specified in the report. GRCh37 human reference genome (GCA_000001405.1) and HGVS variant nomenclature was used for analysis and reporting.

Quantitative real-time RT-PCR analysis is performed to detect mRNA expression of the CEACAM5 gene using primers and probe for the CEACAM5 gene and GUSB housekeeping control gene. CEACAM5 expression at >200 Gene Expression Units (GEU) has a positive predictive value of 86.7% [95%CI: 59.5- 98.3] for prediction of cystic precursor neoplasms (IPMN, MCN, IOPN) in mutation negative samples. CEACAM5 lower limit of detection is 100-200 cells in cyst fluid sample.

The Torrent Suite Software v5.12 and Genomic Classifier (GC) algorithm is used for data analysis. Test results are reported as Negative (low probability of neoplasia) or Positive (high probability of neoplasia). In addition, it reports predicted cyst type and risk of high grade dysplasia or cancer. PancreaSeq GC sensitivity for prediction of cystic precursor neoplasms (IPMN, MCN, IOPN) is 92% [95%CI: 0.86 - 0.96] and specificity 100% [95%CI: 0.92 - 1.00] and for prediction of high grade dysplasia/ cancer is 83% [95%CI: 0.71 - 0.91] and 98% [95%CI: 0.94 - 1.00], correspondingly.

References:

1. Singhi AD et al. Preoperative Next-Generation Sequencing of Pancreatic Cyst Fluid is Highly Accurate in Cyst classification and Detection of Advanced Neoplasia. Gut, 2018; 67:2131-2141.
2. Paniccia A, et al. Prospective, Multi-Institutional, Real-Time Next-Generation Sequencing of Pancreatic Cyst Fluid Reveals Diverse Genomic Alterations That Improve the Clinical Management of Pancreatic Cysts. Gastroenterology, 2023; 164:117-133.
3. Nikiforova MN, et al. A Combined DNA/RNA-based Next-Generation Sequencing Platform to Improve the Classification of Pancreatic Cysts and Early detection of Pancreatic Cancer Arising From Pancreatic Cysts. Annals of Surgery, 2023, ahead of print.

Additional details of DNA sequence variants

Gene	Transcript	Genomic Position
MEN1	NM_130799.2	chr11:64575568GT>G

LOW COVERAGE HOTSPOTS OBSERVED IN THE FOLLOWING GENES

NONE

GROSS DESCRIPTION

1 part(s) labeled with patient name and identifiers received from Johns Hopkins Hospital.

Sample 1: One (1) FNA vial received and labeled with patient name and identifiers

DISCLAIMER

PancreaSeq GC is a diagnostic test that was developed and its performance characteristics determined by the UPMC Molecular and Genomic Pathology laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. PancreaSeq test does not sequence genes in their entirety and mutations outside of mutation hotspots, some insertions and deletions may not be detected. This test does not provide information on germline or somatic status of detected mutations. Certain sample characteristics may result in reduced sensitivity, including low sample cellularity (<100-200 cells), sample heterogeneity, low sample quality, and other causes. The information in this report must be used in conjunction with all relevant clinical information and does not intend to substitute clinical judgment. Decisions on patient care must be based on the independent clinical judgment of the treating physician. A treating physician's decision should not be based solely on this or any other single tests or the information in this report.

Electronically signed out by: