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DNAJB1-PRKACA fusions occur in oncocytic pancreatic and biliary neoplasms and are not specific for fibrolamellar hepatocellular carcinoma

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Abstract

Recently discovered *DNAJB1-PRKACA* oncogenic fusions have been considered diagnostic for fibrolamellar hepatocellular carcinoma. In this study, we describe six pancreatobiliary neoplasms with *PRKACA* fusions, five of which harbor the *DNAJB1-PRKACA* fusion.

All neoplasms were subjected to a hybridization capture-based next-generation sequencing assay (MSK-IMPACT), which enables the identification of sequence mutations, copy number alterations, and selected structural rearrangements involving ≥ 410 genes (n=6) and/or to a custom targeted, RNA-based panel (MSK-Fusion) that utilizes Archer Anchored Multiplex PCR technology and next-generation sequencing to detect gene fusions in 62 genes (n=2). Selected neoplasms also underwent FISH analysis, albumin mRNA in-situ hybridization and arginase-1 immunohistochemical labeling (n=3).

Five neoplasms were pancreatic, and one arose in the intrahepatic bile ducts. All revealed at least focal oncocytic morphology: three cases were diagnosed as intraductal oncocytic papillary neoplasms, and three as intraductal papillary mucinous neoplasms with mixed oncocytic and pancreatobiliary or gastric features. Four cases had an invasive carcinoma component composed of oncocytic cells. Five cases revealed *DNAJB1-PRKACA* fusions and one revealed an *ATP1B1-PRKACA* fusion. None of the cases tested were positive for albumin or arginase-1. Our data prove that *DNAJB1-PRKACA* fusion is neither exclusive nor diagnostic for fibrolamellar hepatocellular carcinoma, and caution should be exercised in diagnosing liver tumors with *DNAJB1-PRKACA* fusions as fibrolamellar hepatocellular carcinoma, particularly if a pancreatic lesion is present. Moreover, considering *DNAJB1-PRKACA* fusions lead to up-regulated protein kinase activity and that this up-regulated protein kinase activity has a significant role in tumorigenesis of fibrolamellar hepatocellular carcinoma, protein kinase inhibition could have therapeutic potential in the treatment of these pancreatobiliary neoplasms as well, once a suitable drug is developed.

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Keywords

DNAJB1; *PRKACA*; fusion; oncocytic; pancreatic; biliary; fibrolamellar hepatocellular carcinoma

INTRODUCTION:

The introduction of routine broad-spectrum genomic analysis of solid neoplasms has generated a wealth of data about alterations at the DNA and RNA level, some of which appear to have diagnostic specificity for distinctive neoplasms, and others may confer sensitivity to targeted therapies. Recently, a novel *DNAJB1-PRKACA* fusion was discovered in the fibrolamellar variant of hepatocellular carcinoma [1]. Both genes are located on the short arm of chromosome 19, and the in-frame fusion occurs due to a 400 kb deletion. *PRKACA* encodes the catalytic subunit of protein kinase A (PKA). In presence of cyclic AMP, the catalytic subunit of PKA is involved in regulation of downstream effectors via phosphorylation. The *DNAJB1-PRKACA* fusion results in the formation of a chimeric protein, which has up-regulated protein kinase activity [2]. It is believed that this up-regulated protein kinase activity has a significant role in tumorigenesis of fibrolamellar hepatocellular carcinoma [3]. The published literature documents the presence of this fusion in >95 % of fibrolamellar hepatocellular carcinomas, but it is reportedly absent in other neoplasms of the liver or other anatomical sites [4]. Thus, identification of this fusion has been regarded as diagnostic for fibrolamellar hepatocellular carcinoma.

However, our comprehensive molecular testing of pancreatobiliary neoplasms demonstrated the recurrent presence of *DNAJB1-PRKACA* fusions in a subset with oncocytic features. Here, we discuss the clinicopathologic and molecular features of these pancreatobiliary neoplasms.

METHODS:

The study was approved by the institutional review board.

Memorial Sloan Kettering Cancer Center institutional database of 34830 solid neoplasms that underwent clinical sequencing testing with MSK-IMPACT (n=33,634) or MSK-Fusion Panel (n=1196) assays as well as the authors' prior research cohorts of 23 pancreatic and biliary intraductal oncocytic papillary neoplasms [5; 6; 7; 8] were searched for cases with fusions involving *DNAJB1* or *PRKACA*, the specific *DNAJB1-PRKACA* fusion in particular. MSK-IMPACT is a hybridization capture-based next generation sequencing assay that assesses the coding regions as well as selected promoter and intronic regions of 410 genes (n=23849) or 468 genes (n=9785), depending upon the version of the test employed, for mutations, amplifications, deletions, selected structural rearrangements (including *DNAJB1-PRKACA* fusions), and microsatellite status against a patient's matched normal [9; 10; 11]. Only cases tested using the newer (01/30/2015 or later) versions of the MSK-IMPACT panel (MSK-IMPACT 410 or 468) were included as the previous versions did not include probes that specifically target DNAJB1 intronic rearrangements. The MSK-Fusion assay is a custom targeted, RNA-based panel that utilizes Archer Anchored Multiplex PCR technology and next-generation sequencing to detect gene fusions in 62 genes (including

DNAJB1, *PRKACA*, and *ATP1B1*) known to be involved in chromosomal rearrangements [12; 13; 14]. These custom assays have been validated and approved for clinical use at Memorial Sloan Kettering Cancer Center by the New York State Department of Health Clinical Laboratory Evaluation Program.

Separately, the Cancer Genome Atlas database [15; 16] was also searched for additional cases with fusions involving these genes.

Histologic sections of the cases with *DNAJB1-PRKACA* fusions in the institutional database were evaluated to characterize the diagnostic features of the neoplasms [slides of the primary neoplasm and metastases (if applicable) were reviewed and tissue from the primary was tested]. Available medical records, including pathology reports, were reviewed to obtain clinical data and outcome.

The cases with *DNAJB1-PRKACA* fusions, for which additional material was available (n=3), underwent FISH analysis following the standard protocols. Briefly, 4 μm-thick tissue sections were de-paraffinized, followed by dehydration in 100% ethanol. Tissues were treated with pepsin for 10-25 minutes, followed by fixation in 10% formalin, and dehydration in a series of 70%, 85% and 100% ethanol. The PRKACA break-apart probe set (Empire Genomics, Buffalo, NY) consists of two BAC probes for the 5'PRKACA region (383kb, labeled in spectrum red) and 3'PRKACA region (542kb, labeled in spectrum green), respectively. After applying the FISH probes to the tissue, both tissue and probes were co-denatured, hybridization was set at 37° C overnight, followed by post-hybridization washing, and counterstained with DAPI. Signal analysis was performed in combination with morphology correlation, and at least 100 interphase cells within the marked tumor area were evaluated and imaged using a Zeiss fluorescence microscope coupled with Metasystems ISIS software (Newton, MA).

The cases with *DNAJB1-PRKACA* fusions, for which additional material was available (n=3), as well as cases of intraductal oncocytic papillary neoplasms of the pancreas and bile ducts from prior research cohorts (n=23) [6; 7; 8] were also labeled with arginase-1 immunohistochemical stain (Cell Marque, Rocklin, CA), using the standard avidin-biotin peroxidase method, and albumin mRNA in-situ hybridization, using the automated ViewRNA platform (Affymetrix) as previously described [17], to assess diagnostic value of arginase and albumin staining in these neoplasms.

RESULTS:

Among the 33,634 cases in the MSK-IMPACT clinical sequencing cohort (2434 of these cases also had MSK-Fusion), we found two non-fibrolamellar hepatocellular carcinoma samples that harbored *DNAJB1-PRKACA* fusions. Both neoplasms were pancreatobiliary primaries with oncocytic morphology, and none had typical histologic features of fibrolamellar hepatocellular carcinoma. Additionally, among the 1196 cases in the MSK-Fusion clinical sequencing cohort (none of these cases had MSK-IMPACT), another pancreatobiliary neoplasm with similar oncocytic morphology was found to harbor an *ATP1B1-PRKACA* fusion. The prior research cohorts of 23 pancreatic and biliary

intraductal oncocytic papillary neoplasms [5; 6; 7; 8] revealed three more cases with *DNAJB1-PRKACA* fusions.

Of note, pancreatobiliary neoplasms in our MSK-IMPACT clinical sequencing cohort included ampullary carcinomas (n=84), pancreatic ductal adenocarcinomas (n=1584), adenosquamous carcinomas of the pancreas (n=36), undifferentiated carcinomas of the pancreas (n=18), intraductal papillary mucinous neoplasms of the pancreas (n=14), mucinous cystic neoplasms of the pancreas (n=3), pancreatic neuroendocrine tumors (n=200), acinar cell carcinomas (n=34), pancreatoblastomas (n=3), solid pseudopapillary neoplasms (n=8), gallbladder carcinomas (n=138), cholangiocarcinomas, NOS (n=56), extrahepatic cholangiocarcinomas (n=102), perihilar cholangiocarcinomas (n=13), and intrahepatic cholangiocarcinomas (n=334). Our MSK-Fusion clinical sequencing cohort included pancreatic ductal adenocarcinomas (n=13), adenosquamous carcinomas of the pancreas (n=2), pancreatic neuroendocrine tumor (n=1), acinar cell carcinomas (n=2), pancreatoblastoma (n=1), gallbladder carcinomas (n=2), cholangiocarcinomas, NOS (n=6), and intrahepatic cholangiocarcinomas (n=4). The prior research cohorts included solely intraductal oncocytic papillary neoplasms of the pancreas (n=13) and bile ducts (n=10).

Of the 10,967 samples in the Cancer Genome Atlas database, none of the non-fibrolamellar hepatocellular carcinoma samples harbored *DNAJB1-PRKACA* fusions. However, there were four samples that harbored *PRKACA* rearrangements with other partner genes and the primary sites of those neoplasms were breast (two invasive ductal carcinomas; one with a *GATA2A-PRKACA* fusion, another with a *TPGS1-PRKACA* fusion), lung (an invasive squamous cell carcinoma with an *ASF1B-PRKACA* fusion), and ovary (a high grade papillary serous carcinoma with an *ASF1B-PRKACA* fusion) [16].

Clinical Features:

The clinicopathologic features of the six *PRKACA* fusion positive oncocytic pancreatobiliary neoplasms are summarized in Table 1. The cohort included five males and one female, and the mean age was 55 years at first presentation (range, 36-76 years). Five cases were pancreatic and one case arose in an intrahepatic bile duct. Follow-up data were available for five patients (interval 9 months - 20 years). One patient died of disease, 4 years after initial diagnosis. One patient had a local recurrence as well as immunohistochemically confirmed distant metastases after 20 years. At the time of last follow-up, the remaining three patients were alive with no evidence of disease 9 months, 6 years, and 10 years after initial diagnosis.

Histologic Features:

There was significant morphologic overlap among these six neoplasms. All six were either an intraductal neoplasm (n=2) or an invasive carcinoma arising in association with an intraductal neoplasm (n=4).

Three cases were diagnosed as intraductal oncocytic papillary neoplasm (two in the pancreas, one in the intrahepatic bile ducts). These cases exhibited arborizing papillae lined by multiple layers of neoplastic cells with abundant eosinophilic, granular cytoplasm and large, and fairly uniform nuclei containing single, prominent nucleoli (Figure 1).

Intraepithelial lumina and interspersed goblet cells were also present [5]. Three additional pancreatic cases were diagnosed as intraductal papillary mucinous neoplasms with mixed oncocytic and pancreatobiliary features (n=2) or mixed oncocytic and gastric features (n=1), as they also demonstrated foci of pancreatobiliary or gastric differentiation (Figure 2).

Four cases had an associated invasive carcinoma component (three in the pancreas, one in the intrahepatic bile ducts). The invasive component was characterized either by small tubular units composed of oncocytic cells infiltrating loose, myxoid stroma or by stromal mucin accumulation in which oncocytic neoplastic cells were suspended, resembling the pattern of colloid carcinoma (Figure 1). In one case, prominent cytoplasmic eosinophilic globules were noted.

Immunohistochemical Features:

None of the three cases with *DNAJB1-PRKACA* fusions tested (Cases #2, #3 and #5) were positive for albumin mRNA by in-situ hybridization or arginase-1 by immunohistochemistry.

Among the 23 cases from the prior research cohorts, although two pancreatic (15%) and one biliary (10%) intraductal oncocytic papillary neoplasms were positive (all patchy) for albumin mRNA by in-situ hybridization (Figure 3), none demonstrated arginase-1 immunolabeling.

Molecular Features:

Of the six cases harboring *PRKACA* fusions, five revealed *DNAJB1-PRKACA* fusions and one (Case #1) revealed an *ATP1B1-PRKACA* fusion. All five cases with *DNAJB1-PRKACA* fusions harbored fusions involving exon 2 or intron 1 of *DNAJB1* with intron 1 or the promoter of *PRKACA.* Case #1 harbored a fusion involving exon 1 of *ATP1B1* with exon 2 of *PRKACA* (Figures 4 and 5, Table 2).

In all three cases tested (Cases #2, #3 and #5), FISH analysis revealed that more than 90% of the tumor cells had a signal pattern of one single signal (green) for the 3’PRKACA region and one to two normal fusion signals (Figure 6). These results are consistent with a complete deletion of the 5’ *PRKACA* region between *PRKACA* and *DNAJB1*, which results in the *DNAJB1-PRKACA* fusion, supporting the results of MSK-IMPACT and MSK-Fusion assays.

Of note, all cases revealed other genomic mutations and/or alterations. In four cases, mutations in key driver genes involved in the MAPK pathway (*KRAS*, *BRAF* and *RAF1*) were identified. Three of these were pancreatic intraductal papillary mucinous neoplasms with either mixed oncocytic and pancreatobiliary (n=2) or mixed oncocytic and gastric (n=1) features that harbored *KRAS G12R, KRAS G12D* or *BRAF V600_K601delinsE* mutations. The last one was a pancreatic intraductal oncocytic papillary neoplasm that had a *RAF1 A150S* mutation. Additionally, the case with distant metastasis had alterations in key cell cycle regulator genes, such as *TP53* (Table 2 and Figure 4).

DISCUSSION:

In this study, we present six pancreatobiliary neoplasms with fusions involving *PRKACA*. All neoplasms at least focally demonstrated oncocytic morphology in their intraductal and/or invasive components. The morphologic overlap of these neoplasms with each other as well as with fibrolamellar hepatocellular carcinoma, which are also defined by fusions involving the *PRKACA* gene, suggests that the oncocytic morphology may be associated with fusions involving this gene.

In 2014, Honeyman et al. reported the *DNAJB1-PRKACA* fusion transcript in fibrolamellar hepatocellular carcinoma [1]. This finding has been validated by subsequent studies and touted as a diagnostic biomarker for this entity [18]. In the seminal study, the authors documented a ~400kb deletion on chromosome 19 resulting in a fusion that either starts in intron 1 or exon 2 of *DNAJB1* and ends in intron 1 of *PRKACA*. Since the description of the fusion, several studies have been undertaken to determine the role of this fusion transcript in the development of fibrolamellar hepatocellular carcinoma. The fusion codes for an active and oncogenic form of protein kinase A (PKA) enzyme. The fusion protein is phosphorylated at a site in the Cα catalytic subunit of the PKA (PKA-Cα), which is often associated with kinase activity even in the absence of activators of adenylyl cyclase, suggesting that the fusion protein is constitutively more active than wild-type PKA-Cα, but can be further induced with signals that normally activate PKA in cells [19]. The reason for upregulated activity of the fusion transcript as compared to wild-type PKA may be related to the replacement of the *PRKACA* promoter by the *DNAJB1* promoter, leading to a higher basal transcription rate. Engelholm et al., using CRISPR/Cas9 techniques, showed generation of the *DNAJB1-PRKACA* fusion gene in wild-type mice to be sufficient to initiate formation of tumors that have many features of human fibrolamellar hepatocellular carcinoma, but carcinogenesis may be more complex in humans [20]. In fact, in human fibrolamellar hepatocellular carcinomas, recurrent mutations that hyperactivate the Wnt pathway have been reported, together with the *DNAJB1-PRKACA* fusion. Furthermore, genetic alteration of this pathway -but not several other oncogenes or tumor suppressors- synergized with *DNAJB1-PRKACA* driven carcinogenesis in mouse models [21]. Sanford et al. also suggested that sole activation of *PRKACA* is not sufficient for human carcinogenesis and perhaps the conformational properties of this chimera may play a role [22]. More recently, microRNA-375 dysregulation was also identified in cases of fibrolamellar hepatocellular carcinoma, but how the *DNAJB1-PRKACA* fusion transcript plays a role in the suppression of microRNA-75 expression and whether microRNA-75 has important targets in this tumor remain to be studied [23]. Of note, while the *DNAJB1-PRKACA* fusion is highly recurrent in fibrolamellar hepatocellular carcinoma, rare cases of fibrolamellar hepatocellular carcinoma without the fusion have been described [2, 24]. In those cases, alternative mechanisms of upregulation of PKA, such as loss of *PRKACA1* in Carney complex, or amplification of *PRKACA,* have been demonstrated [4, 24, 25].

Through analysis of a large series of pancreatobiliary neoplasms, we identified six neoplasms, five with *DNAJB1-PRKACA* fusions and one with an *ATP1B1-PRKACA* fusion. The structural variants of *DNAJB1-PRKACA* observed in our study are similar to those described by Honeyman et al in their seminal study [1], indicating that the functional

implications of the fusions are also most likely similar [1]. However, additional possible driver mutations, including key drivers of the *MAPK* pathway and key cell cycle regulatory genes, were also present in our cases (Table 2). Of note, *ATP1B1-PRKACA* fusions have been reported in cholangiocarcinoma [26, 27]. *ATP1B1* is also a known gene partner in *NRG1* rearrangements, which are oncogenic drivers that are enriched in invasive mucinous adenocarcinomas of the lung [28].

In addition to the shared genomic event, these pancreatobiliary neoplasms shared some specific histologic features as well. All six cases had an intraductal neoplasm with at least mixed, if not pure, oncocytic morphology. Of note, the pancreatic intraductal papillary mucinous neoplasms with mixed (oncocytic and pancreatobiliary or gastric) features harbored MAPK pathway mutations, more typical of intraductal papillary mucinous neoplasms [6]. All four cases with an invasive component also revealed similar oncocytic features in the invasive carcinoma. The morphologic overlap between fibrolamellar hepatocellular carcinomas and the pancreatobiliary neoplasms in this series is also interesting. Fibrolamellar hepatocellular carcinomas are composed of cords of large and polygonal neoplastic cells in background of dense collagen bundles frequently arranged in parallel lamellae. The neoplastic cells have abundant granular and eosinophilic cytoplasm with frequent hyaline globules and typical nuclear features include open chromatin and prominent “cherry red” macronucleoli [4]. While the pancreatobiliary neoplasms in this series are architecturally different from fibrolamellar hepatocellular carcinoma, they are cytologically similar, as they also exhibit abundant granular and eosinophilic cytoplasm, large vesicular nuclei, and prominent nucleoli [29].

Although number of the cases is too limited to draw any definitive conclusions, the morphologic similarity among these tumors that have the same fusion resulting in activation of PKA is intriguing. Studies have shown that oncocytic neoplasms including fibrolamellar hepatocellular carcinoma and intraductal oncocytic papillary neoplasm are rich in mitochondria [30]. It is also known that PKA acts on several substrates located in the mitochondria in various organs [31]. The constitutional activation of the PKA pathway by the chimeric fusion protein probably causes mitochondrial hyperplasia within the cells, which results in the oncocytic appearance. Therefore, it is possible that fibrolamellar hepatocellular carcinoma and the pancreatobiliary neoplasms described in this series may share common progenitors, especially considering they overlap not only morphologically but also immunophenotypically. While conventional hepatocellular carcinomas are usually CK7 negative, fibrolamellar hepatocellular carcinomas label with CK7 [32; 33] and oncocytic pancreatobiliary neoplasms express HepPar-1 [7; 34] and, focally, albumin mRNA. Moreover, transcriptomic analysis of fibrolamellar hepatocellular carcinomas has shown enrichment of certain transcription factor gene sets associated with pancreatic cancer, namely *ERBB2, E2F1, E2F3, CDKN2A, CDK6, SMAD2, TGFBR1, and TGFB2* [3]. What this likely means is that altered PKA function, leading to alteration in the activity of these oncogenic proteins, may at least play a part in, if not driving, oncogenesis. Interestingly, one of our cases shared genomic mutations/alterations in some of the above-mentioned genes (*ERBB2* in Case #6).

Our data raise awareness that *DNAJB1-PRKACA* fusions are not unique to fibrolamellar hepatocellular carcinoma and may also be seen in oncocytic pancreatobiliary neoplasms. Although diagnostic confusion is unlikely based upon resected specimens, caution should be exercised in the context of biopsy and cytology specimen interpretation. Because the differential diagnosis of an oncocytic neoplasm in the liver, perihepatic soft tissue or regional lymph nodes, with CK7, HepPar-1, and albumin mRNA expression and a *DNAJB1-PRKACA* fusion includes fibrolamellar hepatocellular carcinoma as well as intraductal oncocytic papillary neoplasms (and associated invasive carcinomas) of the bile ducts and the pancreas [32; 35]. Therefore, more specific hepatocellular differentiation markers, such as arginase-1 immunohistochemical staining, as well as radiographic findings demonstrating the lack of a pancreas mass may be needed to establish a definitive diagnosis.

Furthermore, this chimera is potentially targetable, and new therapeutics are on the horizon, including kinase inhibitors or other modalities altering the PKA pathway [36]. Considering clinical trials using non-specific tyrosine/aurora kinase inhibitors for advanced stage fibrolamellar hepatocellular carcinoma are already enrolling patients [37], it is only a matter of time before specific targeted therapy becomes available for fibrolamellar hepatocellular carcinoma. Identifying *PRKACA* fusions will then become therapeutically important not only for fibrolamellar hepatocellular carcinoma but also in other neoplasms harboring this fusion [38].

Conclusion:

We report six novel cases of oncocytic pancreatobiliary neoplasms with *PRKACA* fusions, including five with *DNAJB1-PRKACA* fusions. Our data proves that *DNAJB1-PRKACA* fusion is not a specific marker for the diagnosis of fibrolamellar hepatocellular carcinoma. As in depth next-generation sequencing of tumors becomes a standard of care in the oncologic management of patients, we are likely to find more cases exhibiting similar molecular alterations, which might be amenable to targeted therapy.

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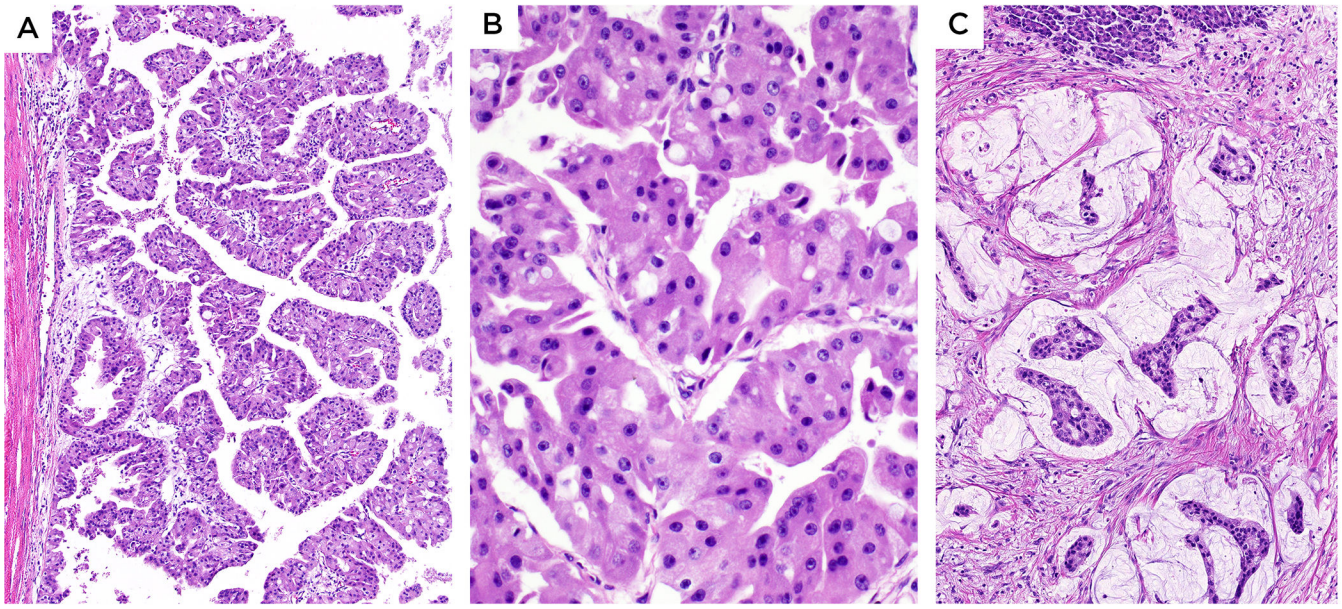

Figure 1:

A-B. Intraductal oncocytic papillary neoplasms exhibited papillary architecture with distinct oncocytic cytology and intracytoplasmic lumens. **C.** If present, invasive component revealed stromal mucin accumulation in which the neoplastic cells were suspended (Case 1 is depicted here).

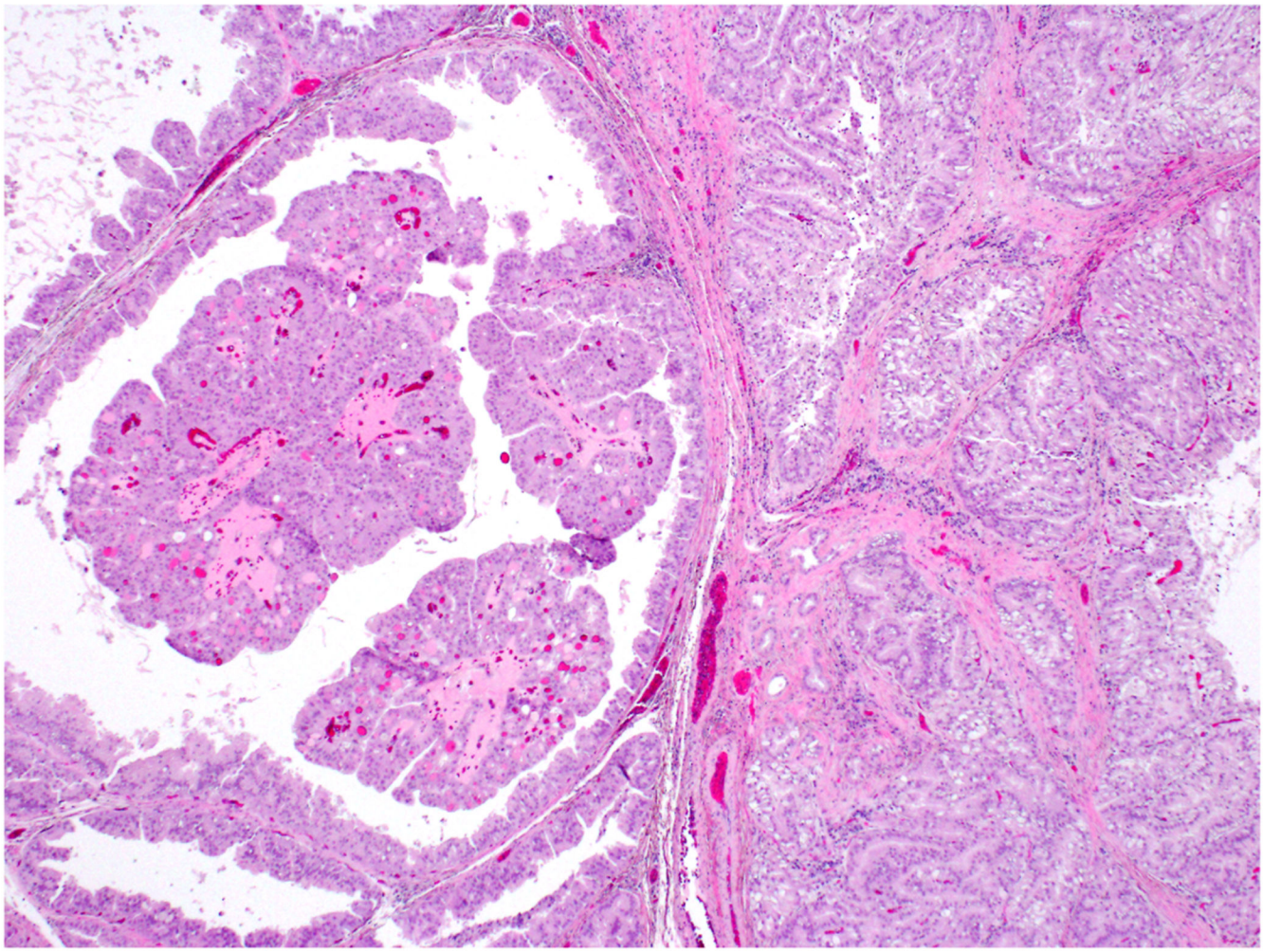

Figure 2:
Three pancreatic neoplasms demonstrated mixed features, Case 5 with mixed oncocytic and gastric features is shown here.

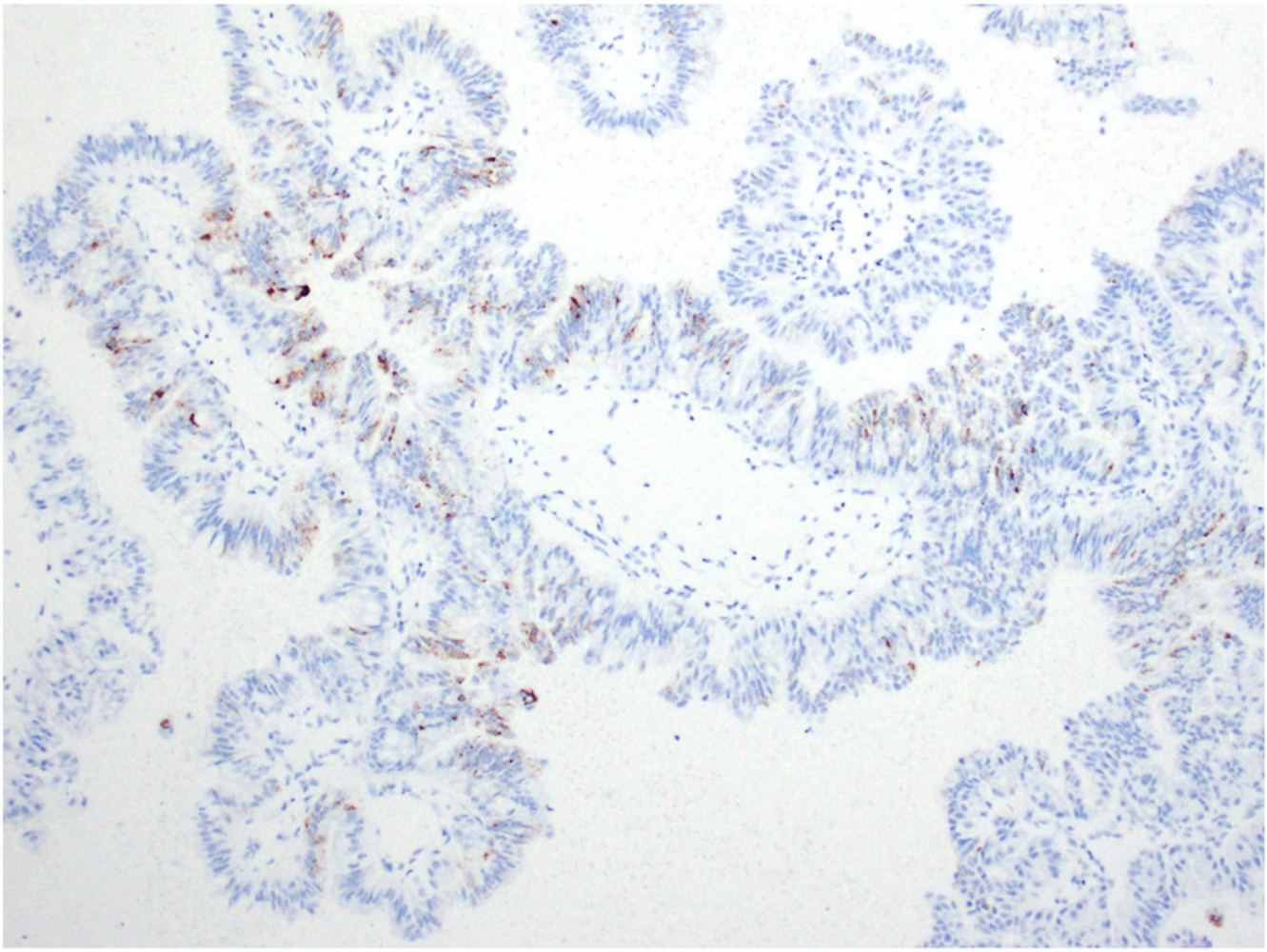

Figure 3:
Albumin mRNA by in-situ hybridization was positive in two pancreatic intraductal oncocytic papillary neoplasms (none of these cases had *DNAJB1-PRKACA* fusions).

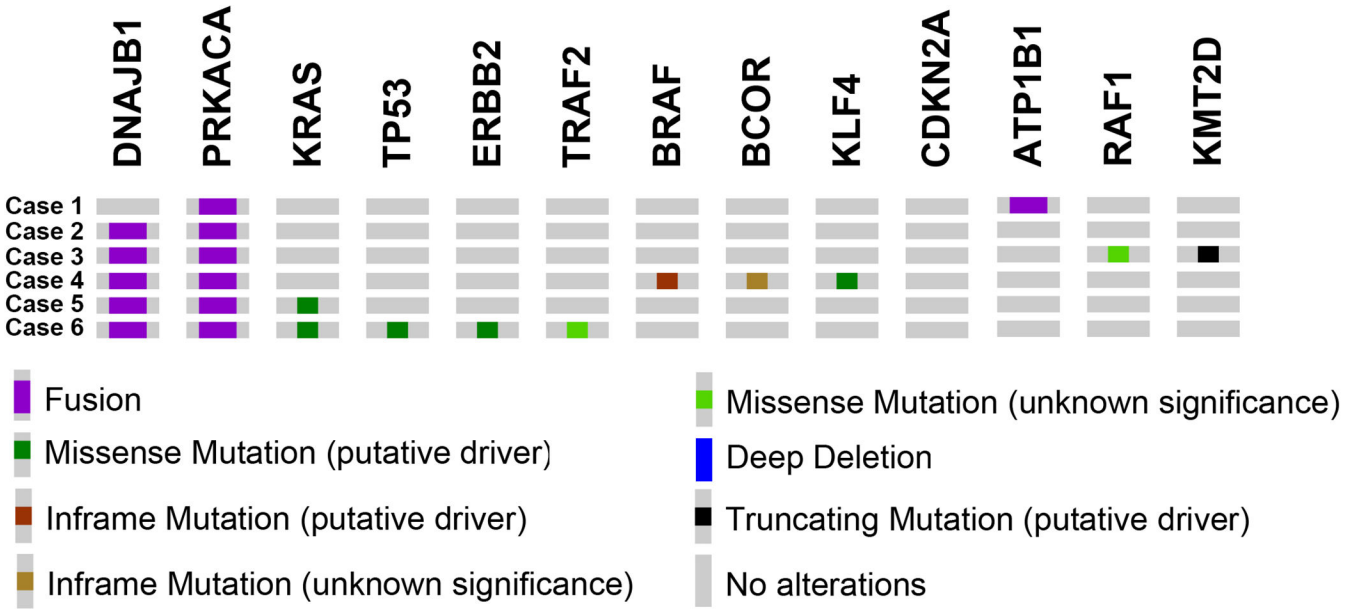

Figure 4:
OncoPrint diagram of types of genetic alterations seen in our cases.

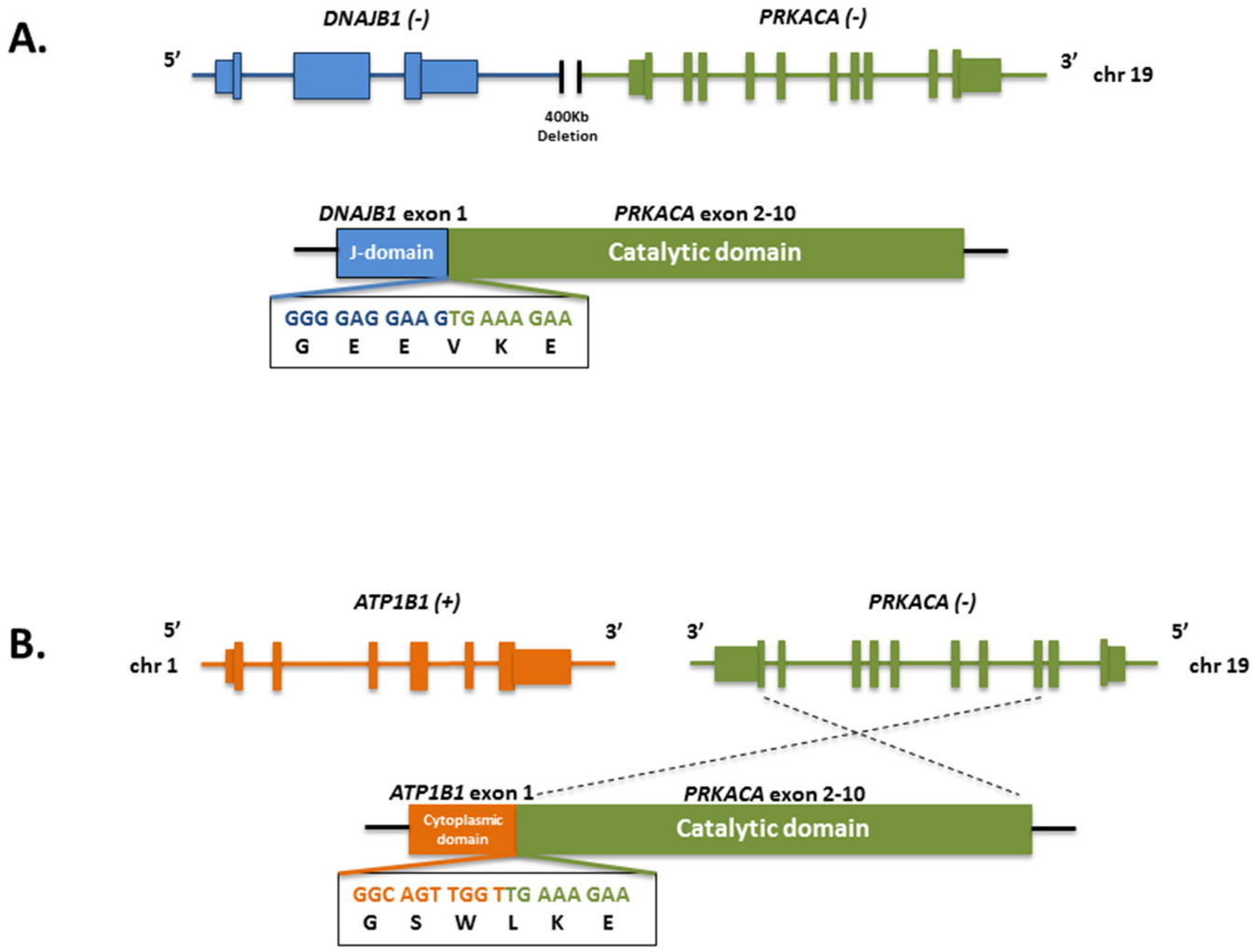

Figure 5:

Schematic representations of *PRKACA* fusions detected by the MSK-Fusion targeted RNASeq assay. **A.** *DNAJB1-PRKACA* in-frame fusion resulting from a 400 Kb deletion on chromosome 19 and joining exon1 of *DNAJB1* (NM_006145) to exons 2-10 of *PRKACA* (NM_002730). **B.** *ATP1B1-PRKACA* fusion derived from a translocation between chromosome 1 and chromosome 19. This in-frame fusion event involves exon 1 of *ATP1B1* (NM_001677) and exons 2-10 of *PRKACA* (NM_002730). The chimeric transcript sequence and its corresponding protein sequence are indicated under the fusion breakpoint region. +/− indicate the direction of transcription of each gene.

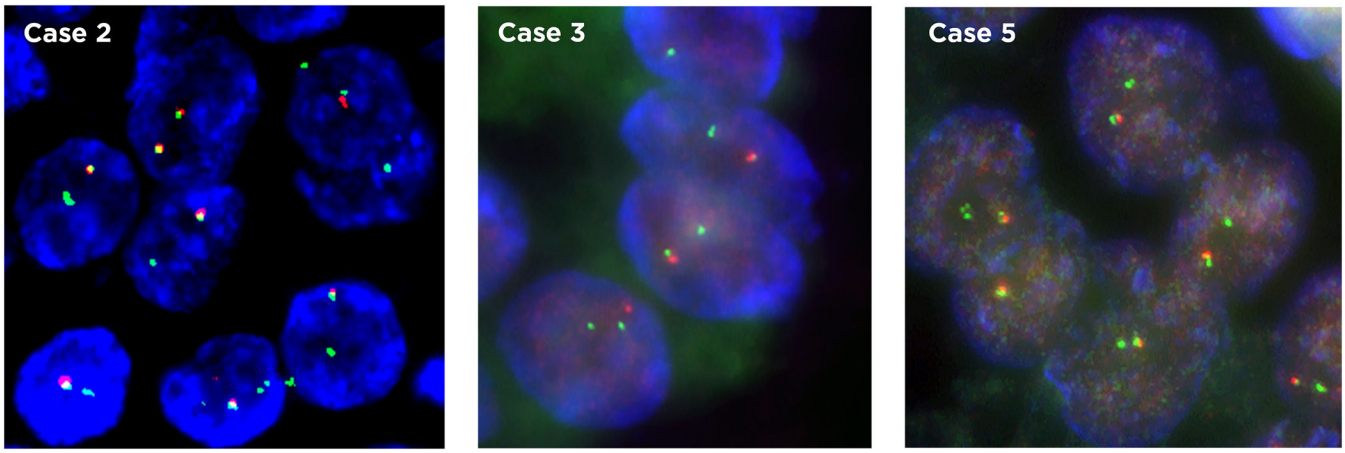

Figure 6:

FISH analysis using PRKACA break-apart probes reveals a complete loss of the 5'PRKACA signal (labeled in red) in most cells, indicative of an interstitial deletion of the 5' upstream region between the *PRKACA* and *DNAJB1* at 19p13.12.

Table 1:

Clinicopathologic Features of the Pancreatobiliary Neoplasms with *PRKACA* Fusions

Case	Age/Sex	Initial Presentation	Pathology Diagnosis			Clinical Follow-up
			Intraductal Component	Invasive Component	Metastasis	
1	63/M	7 cm mass in head of the pancreas	IOPN	Invasive adenocarcinoma with mucinous and oncocytic features	Not applicable	No follow up data available
2	47/M	10 cm cystic mass in caudate lobe of liver	IOPN of the bile ducts	Invasive adenocarcinoma with oncocytic features	Not applicable	Recurrence after 4 years; now deceased
3	36/M	4.5 cm cystic mass in head of the pancreas	IOPN	Not applicable	Not applicable	No evidence of disease for 10 years
4	58/F	Complex cystic mass in head of the pancreas	IPMN, mixed oncocytic and pancreatobiliary features	Minute focus of invasion with mucinous and oncocytic features	Not applicable	No adjuvant therapy given. No evidence of disease for 9 months
5	52/M	2.8 cm cystic mass in head of the pancreas	IPMN, mixed oncocytic and gastric features	Not applicable	Not applicable	No evidence of disease for 6 years
6	76/M	Distal pancreatic mass	IPMN, mixed oncocytic and pancreatobiliary features	Invasive adenocarcinoma	Metastatic adenocarcinoma with oncocytic features, involving liver	Recurrence and liver metastases after 20 years, disease progression despite chemotherapy

IOPN: Intraductal oncocytic papillary neoplasm; IPMN: Intraductal papillary mucinous neoplasm

Table 2:

Molecular Features of *PRKACA* Rearranged Pancreatobiliary Neoplasms

Case	Diagnosis	Test	Partner Gene (5')	Kinase Gene (3')	Predicted Fusion	Other Genomic Mutations / Alterations
1	IOPN with invasion	MSK-Fusion	*ATP1B1* Exon 1 (NM_001677)	*PRKACA* Exon 2 (NM_002730)	Protein fusion: in frame (*ATP1B1-PRKACA*)	*BCL10 D139V, GREM1 S137del, MYD88 S129C, MDM2* amplification
2	Biliary IOPN with invasion	MSK-IMPACT & FISH	*DNAJB1* Intron 1 (NM_006145)	*PRKACA* Intron 1 (NM_002730)	Protein fusion: in frame (*DNAJB1-PRKACA*)	*KIT P34L, NOTCH4 R373W, BLM D239N, LATS2 V367I*
3	IOPN	MSK-IMPACT & FISH	*DNAJB1* Intron 1 (NM_006145)	*PRKACA* Promoter (NM_002730)	Transcript fusion (*DNAJB1-PRKACA*)	*RAF1 A150S, KMT2D G5189R, KMT2D W2038X*
4	IPMN, mixed oncocytic and pancreatobiliary features, with invasion	MSK-IMPACT	*DNAJB1* Intron 1 (NM_006145)	*PRKACA* Intron 1 (NM_002730)	Protein fusion: in frame (*DNAJB1-PRKACA*)	*BRAF V600_K601delinsE, BCOR E382Qfs*53, KLF4 K409Q,* loss of chromosome arm 9p21-24
5	IPMN, mixed oncocytic and gastric features	MSK-IMPACT & FISH	*DNAJB1* Exon 2 (NM_006145)	*PRKACA* Intron 1 (NM_207518)	Protein fusion: mid-exon (*DNAJB1-PRKACA*)	*KRAS G12D*
6	IPMN with mixed oncocytic and pancreatobiliary features, with invasion	MSK-IMPACT	*DNAJB1* Intron 1 (NM_006145)	*PRKACA* Intron 1 (NM_002730)	Protein fusion: in frame (*DNAJB1-PRKACA*)	*KRAS G12R, TP53, R110C, ERBB2, R678W, TRAF2 I376V*

IOPN: Intraductal oncocytic papillary neoplasm; IPMN: Intraductal papillary mucinous neoplasm