Am J Surg Pathol • Volume 42, Number 12, December 2018

Recurrent Mutations in *APC* and *CTNNB1* and Activated Wnt/β-catenin Signaling in Intraductal Papillary Neoplasms of the Bile Duct

A Whole Exome Sequencing Study

Kohei Fujikura, MD, PhD, Masayuki Akita, MD, PhD,*† Tetsuo Ajiki, MD, PhD,†*
Takumi Fukumoto, MD, PhD,† Tomoo Itoh, MD, PhD, and Yoh Zen, MD, PhD, FRCPath**

汇 报 人 徐婉妮

Intraductal papillary neoplasm of the bile duct (IPNB)

- A distinct type of tumor with unique clinicopathologic features
  - Affected bile ducts are often extensively dilated, and, thus, mimic cystic neoplasms
  - Mucin overproduction
  - Biliary papilloma(tosis)
  - Mucin-producing bile duct tumors
  - Biliary cystadenocarcinomas with no ovarian-like stroma
- Originally used by Chen et al. in 2001 to describe hepatolithiasis-associated biliary tumours

Intraductal papillary neoplasm of the bile duct (IPNB)

- The 2010 World Health Organization classification states that ‘IPNB is characterized by dilated bile ducts filled with a non-invasive papillary or villous biliary neoplasm covering delicate fibrovascular stalks’
- Premalignant lesions
  - Intraductal papillary neoplasm with low-or intermediate-grade intraepithelial neoplasia 8503/0
  - Intraductal papillary neoplasm with high-grade intraepithelial neoplasia 8503/2
- Maligant
  - Intraductal papillary neoplasm with an associated invasive carcinoma 8503/3

Intraductal papillary neoplasm of the bile duct (IPNB)

- Currently regarded as a biliary counterpart of pancreatic IPMNs
- 4 subtypes: Pancreaticobiliary， Intestinal，Gastric， Oncocytic
- More common in East Asia than in Western countries
  - Hepatolithiasis
  - Clonorchiasis

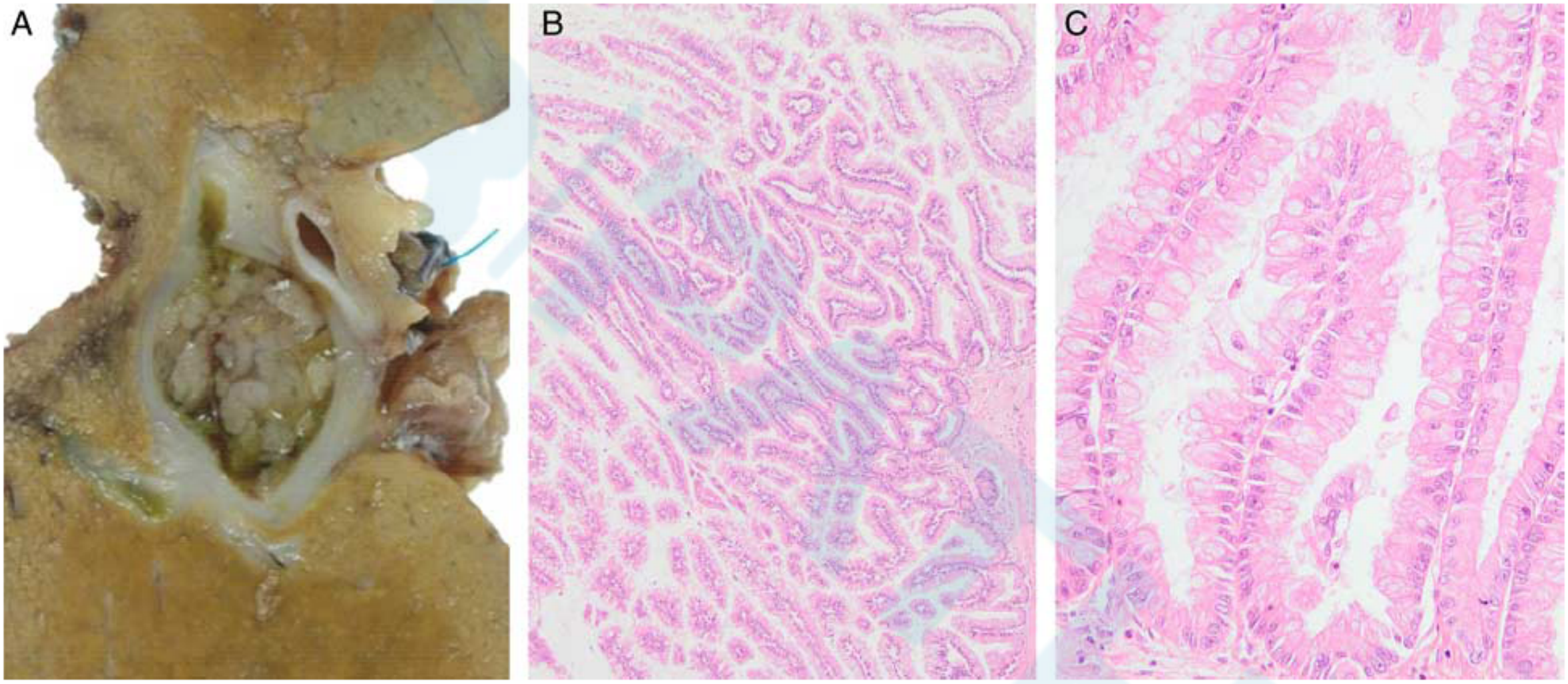
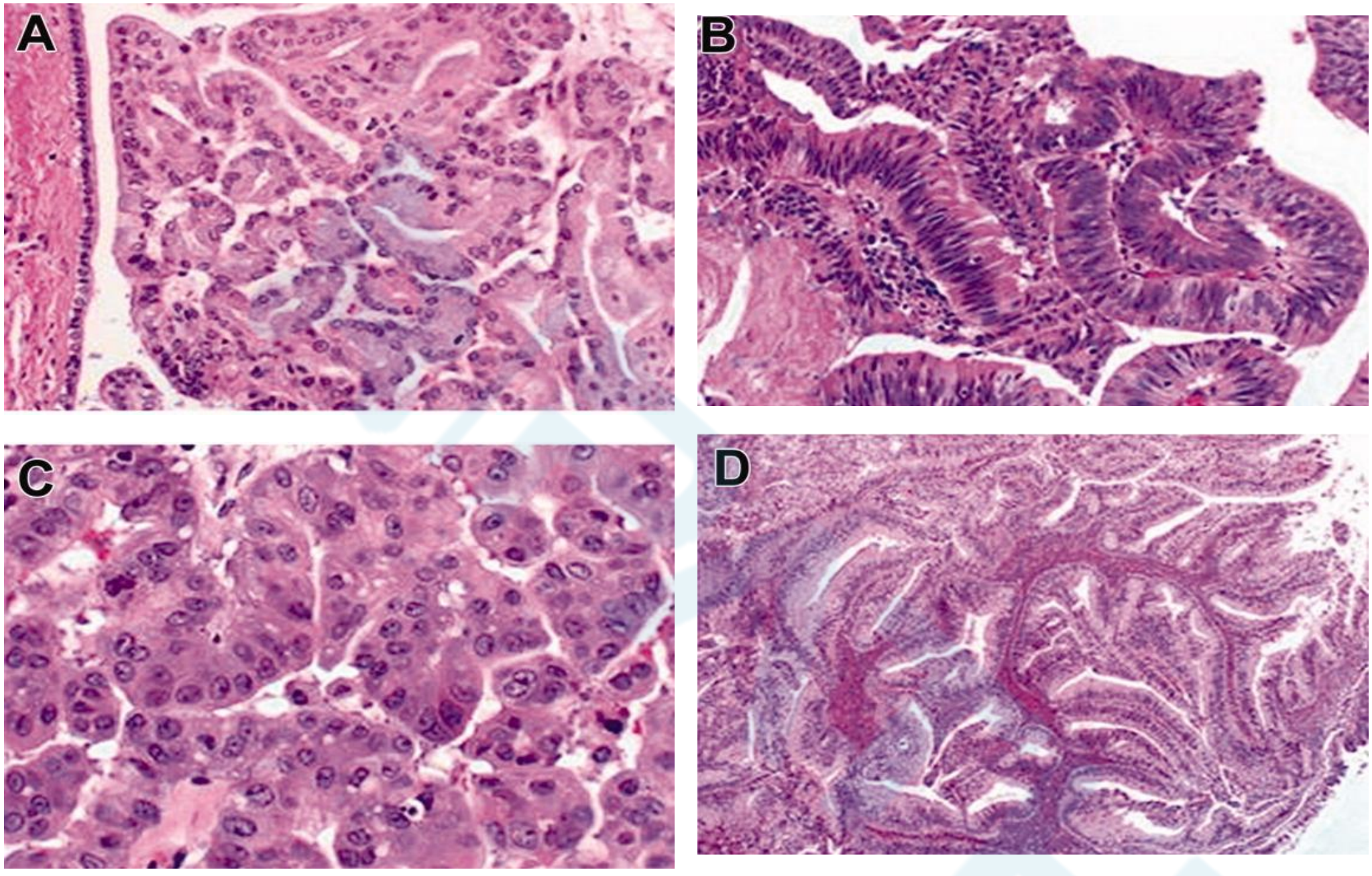


FIGURE 1. Pathologic features of IPNB. A, This case grossly showing intraductal papillary growth with abundant mucin in the dilated intrahepatic bile duct. B, Histologically, tumor cells are arranged in a well-organized papillary architecture with thin fibrovascular stalks. C, Tumor cells have intracytoplasmic mucus and enlarged nuclei with slightly irregular nuclear membrane.



Histologic appearance of epithelial subtypes of IPNB (magnification ×200). (A) Gastric-type epithelium resembling pyloric glands. (B) Intestinal-type epithelium resembling colonic adenocarcinoma with elongated, stratified, and hyperchromatic nuclei. (C) Oncocytic-type epithelium consisting of relatively uniform cells with abundant eosinophilic cytoplasm and centrally located nuclei with prominent nucleoli was associated with occasional intraepithelial mucin-containing lumen formation. (D) Pancreatobiliary-type epithelium resembling monolayered malignant biliary epithelium with marked nuclear pleomorphism and high nuclear-to-cytoplasmic ratio.

Intraductal papillary neoplasm of the bile duct: a biliary equivalent to intraductal papillary mucinous neoplasm of the pancreas? Hepatology. 2012;56:1352–1360.

Papillary cholangiocarcinomas

- Traditionally been used to describe these cases：
  - Intraductal papillary growth, particularly in the extrahepatic bile duct
  - More complex histologic architectures
  - Shared clinicopathologic features with nonpapillary cholangiocarcinomas
- Whether all cases previously called papillary cholangiocarcinoma belong to the spectrum of IPNBs?

Histopathology

Histopathology 2016, **69**, 950–961. DOI: 10.1111/his.13037

Comparative clinicopathological study of biliary intraductal papillary neoplasms and papillary cholangiocarcinomas

Kohei Fujikura,[1] Takumi Fukumoto,[2] Tetsuo Ajiki,[2] Kyoko Otani,[1] Maki Kanzawa,[1] Masayuki Akita,[2] Masahiro Kido,[2] Yonson Ku,[2] Tomoo Itoh[1] & Yoh Zen[1]

[1]*Department of Diagnostic Pathology, and* [2]*Department of Hepato-Biliary-Pancreatic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan*

Date of submission 2 February 2016
Accepted for publication 9 July 2016
Published online *Article Accepted* 13 July 2016

Fujikura K, Fukumoto T, Ajiki T, Otani K, Kanzawa M, Akita M, Kido M, Ku Y, Itoh T & Zen Y (2016) *Histopathology* **69**, 950–961. DOI: 10.1111/his.13037

Comparative clinicopathological study of biliary intraductal papillary neoplasms and papillary cholangiocarcinomas

Aims: The aim of this study was to achieve a better definition of intraductal papillary neoplasms of the bile duct (IPNBs).

Methods and results: Biliary tumours that showed predominantly intraductal papillary growth were provisionally classified as IPNBs ($n = 25$) and papillary cholangiocarcinomas ($n = 27$). IPNB was defined as a neoplasm that is confined to the epithelium or is regularly arranged in a high-papillary architecture along thin fibrovascular stalks, whereas the term 'papillary cholangiocarcinoma' was used for tumours with more complex papillary structures (e.g. irregular papillary branching or mixed with solid–tubular growth). In our consecutive cohort of biliary neoplasms, 5% were classified as IPNBs, and 10% as papillary cholangiocarcinomas. IPNBs differed from papillary cholangiocarcinomas by less advanced invasion, gross mucin overproduction (72% versus 7%), and their prevalent location (84% of IPNBs in intrahepatic/hilar ducts; 70% of papillary cholangiocarcinomas in extrahepatic ducts). Gastric-type and oncocytic-type tumours were only detected in IPNBs. Expression of mucin core proteins and cytokeratin 20 significantly differed between the two groups. *KRAS* and *GNAS* were wild-type genotypes in all but one case of *KRAS*-mutated IPNB. Patients with IPNB had better recurrence-free survival than those with papillary cholangiocarcinoma ($P = 0.007$). In multivariate analysis, in which several other prognostic factors (e.g. stromal invasion and lymph node metastasis) were applied, the classification of the two papillary tumours was an independent prognostic factor ($P = 0.040$).

Conclusions: Given the significant contrast in clinicopathological features between IPNBs and papillary cholangiocarcinomas, it may be more appropriate to use the diagnostic term 'IPNB' for selected tumours that show regular papillary growth, separately from papillary cholangiocarcinomas.

Keywords: bile duct cancer, *GNAS*, intraductal papillary neoplasm, *KRAS*, papillary cancer

	IPNBs	Papillary cholangiocarcinomas
Prevalent site	intrahepatic or hilar bile ducts	extrahepatic bile ducts.
Incidence of gross mucin	>70%	<10%
Morphological type of tumour cells	pancreaticobiliary, intestinal, gastric, oncocytic types	intestinal-type and pancreatobiliary-type
Pathological features	confined to the epithelium, regularly arranged in a high-papillary architecture along thin fibrovascular stalks	irregular papillary branching or mixed with solid–tubular growth
Immunohistochemical analysis	MUC6+, CK20-	MUC6-, CK20+
KRAS and GNAS mutions	KRAS and GNAS were wild-type genotypes	extremely uncommon
Recurrence-free postoperative survival	better	

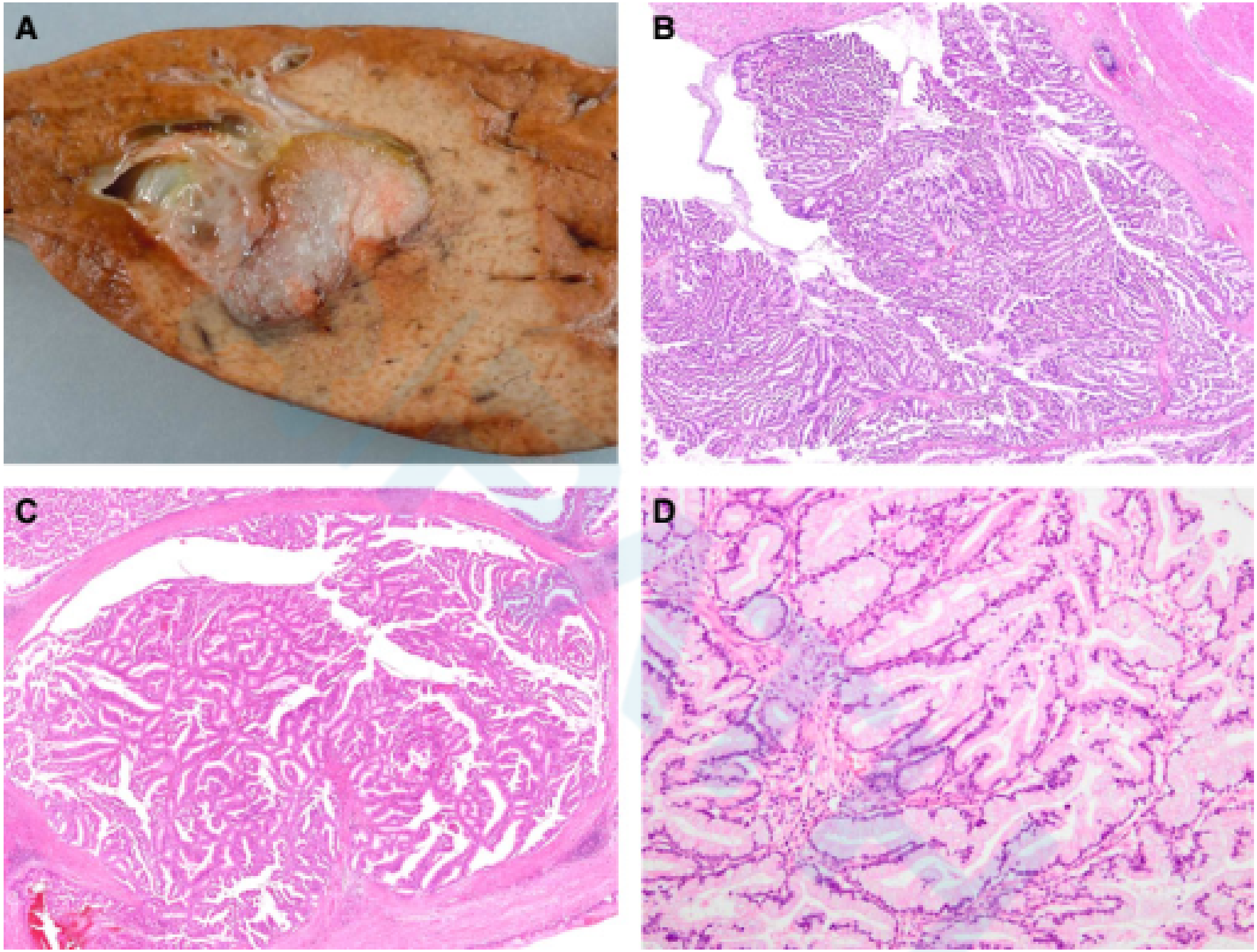


Figure 1. Pathological features of intraductal papillary neoplasms of the bile duct. A, The dilated intrahepatic bile duct is filled with an intraductal papillary neoplasm. B,C, The papillary architecture is regularly organized and relatively uniform in appearance. D, Tumour cells are arranged along thin fibrovascular stalks.

Histopathology 2016, **69**, 950–961. DOI: 10.1111/his.13037

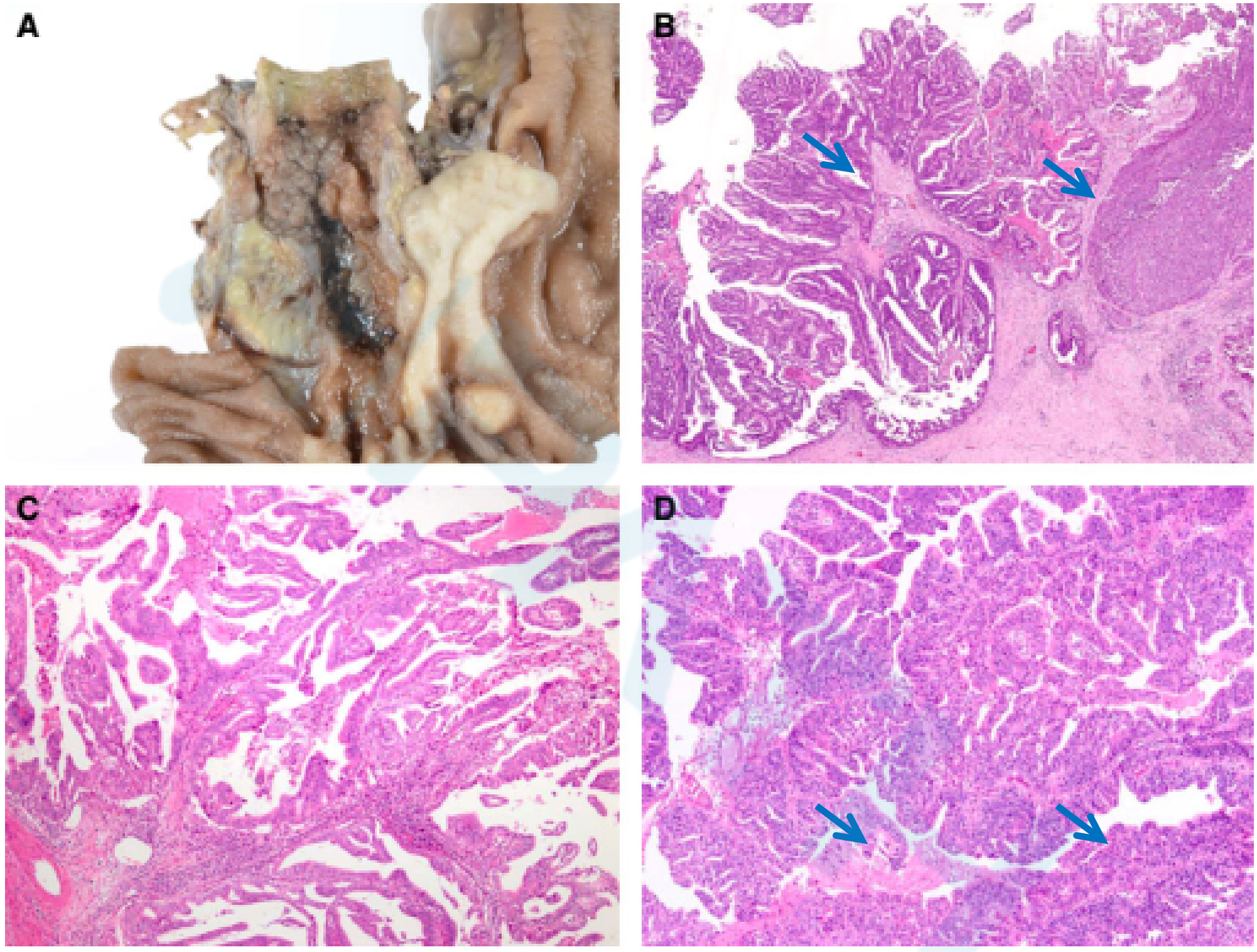


Figure 2. Pathological features of papillary cholangiocarcinomas. A, The tumour is grossly papillary with small granular changes in the adjacent mucosa. The appearance is more solid than that of intraductal papillary neoplasms of the bile duct. B–D, Papillary structures are complex with tubular or cribriform foci. A solid pattern (B) and micropapillary growth (D) are also present. The papillary stroma is variable in thickness.

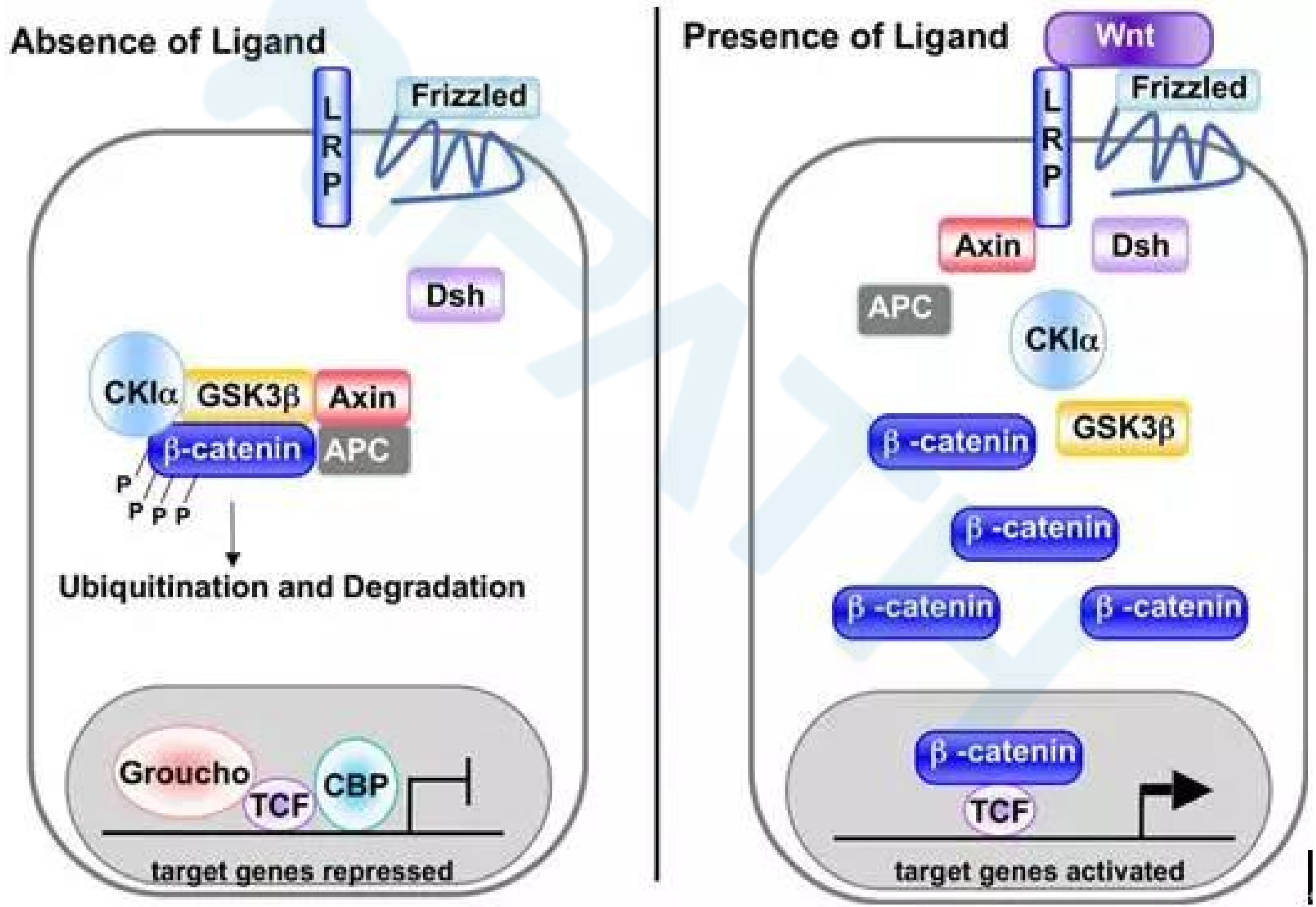
Histopathology 2016, **69**, 950–961. DOI: 10.1111/his.13037

Papillary cholangiocarcinomas

- Given the significant contrast in clinicopathological features between IPNBs and papillary cholangiocarcinomas, it may be more appropriate to use the diagnostic term ‘IPNB’ for selected tumours that show regular papillary growth, separately from papillary cholangiocarcinomas

Histopathology 2016, **69**, 950–961. DOI: 10.1111/his.13037

Wnt/ β -catenin signaling pathway



Intraductal papillary neoplasm of the bile duct (IPNB)

- Elucidate the genetic landscape of biliary papillary neoplasms
  - Previous studies on IPNBs identified mutations in *GNAS, KRAS*, and *RNF43*, the obtained results were not consistent
- Genetic features were first compared
  - IPNBs
  - Papillary cholangiocarcinomas
  - Nonpapillary cholangiocarcinomas

MATERIALS AND METHODS

- 28 cases of biliary papillary neoplasms（14 IPNBs and 14 papillary cholangiocarcinomas）
  - 7 IPNBs exome sequencing studies
  - 21 Sanger sequencing
- 29 cases of nonpapillary cholangiocarcinomas
  - Sanger Sequencing
    - *CTNNB1* (exons 3 and 7) *APC* (exon 15) *KRAS*(exons 2 and 3) *GNAS* (exon 8) *BRAF* (exon 15)

MATERIALS AND METHODS

- Statistical Analysis
  - χ 2 or Fisher test
  - Kaplan-Meier method
  - Log-rank test
- Immunohistochemistry for β-catenin
  - 0 (negative)
  - 1+ (focal), 1% to 5%
  - 2+ (moderate), 6% to 50%
  - 3+ (marked), >50%

TABLE 1. Clinicopathologic Summary of Cases Examined

	Biliary Papillary Neoplasms (n [%])*			
	Total (N = 28)	IPNBs† (N = 14)	Papillary Cholangiocarcinomas (N = 14)	Nonpapillary Cholangiocarcinomas (N = 29) (n [%])
Median age (y)	68.5 (30-84)	68 (30-83)	70 (59-84)	71 (48-82)
Sex (% of males)	64	57	71	45
Tumor location				
Intrahepatic duct	8 (29)	8 (57)	0	6 (21)
Hilar duct	7 (25)	5 (36)	2 (14)	12 (41)
Distal duct	13 (46)	1 (7)	12 (86)	11 (38)
Median size (range; mm)	33.5 (10-115)	28 (10-115)	39 (20-80)	30 (13-71)
Histologic differentiation				
Well and moderately	27 (96)	14 (100)	13 (93)	23 (79)
Poorly	1 (4)	0	1 (7)	6 (21)
Histologic type				
Pancreatobiliary	14 (50)	6 (43)	8 (57)	29 (100
Intestinal	6 (21)	0	6 (43)	0
Gastric	5 (18)	5 (36)	0	0
Oncocytic	3 (11)	3 (21)	0	0
Degree of invasion				
Noninvasive	11 (39)	11 (79)	0	1 (3)
Microinvasion (< 2 mm)	5 (18)	1 (7)	4 (28)	0
Within the duct wall	5 (18)	0	5 (36	8 (28)
Beyond the duct	7 (25)	2 (14)	5 (36)	20 (69)
Components of invasive mucinous carcinoma‡	1 (6)	1 (33)	0	0
Lymph node metastasis	4 (14)	1 (7)	3 (21)	9 (31)
5-year survival (%)	65	89	46	20

*Meeting the current WHO definition of IPNBs.
†Fulfilling the stringent criteria of IPNBs.[10]
‡Only invasive cases examined.

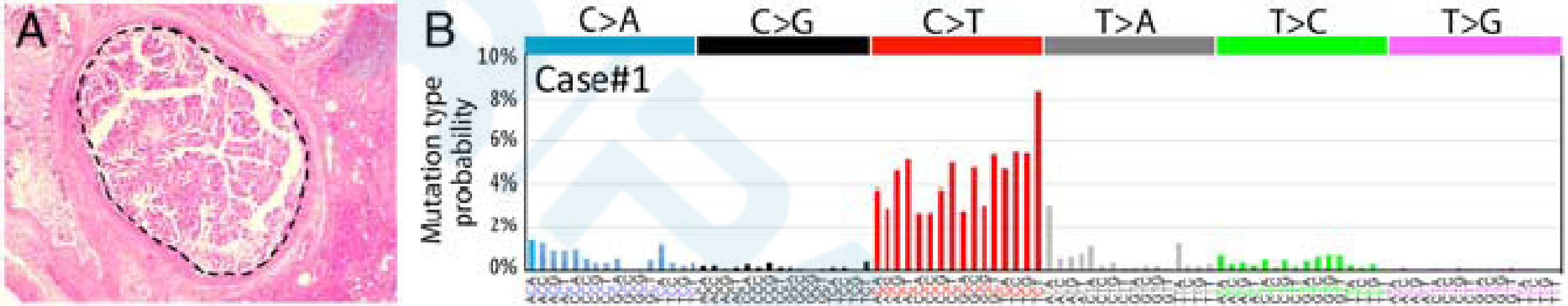
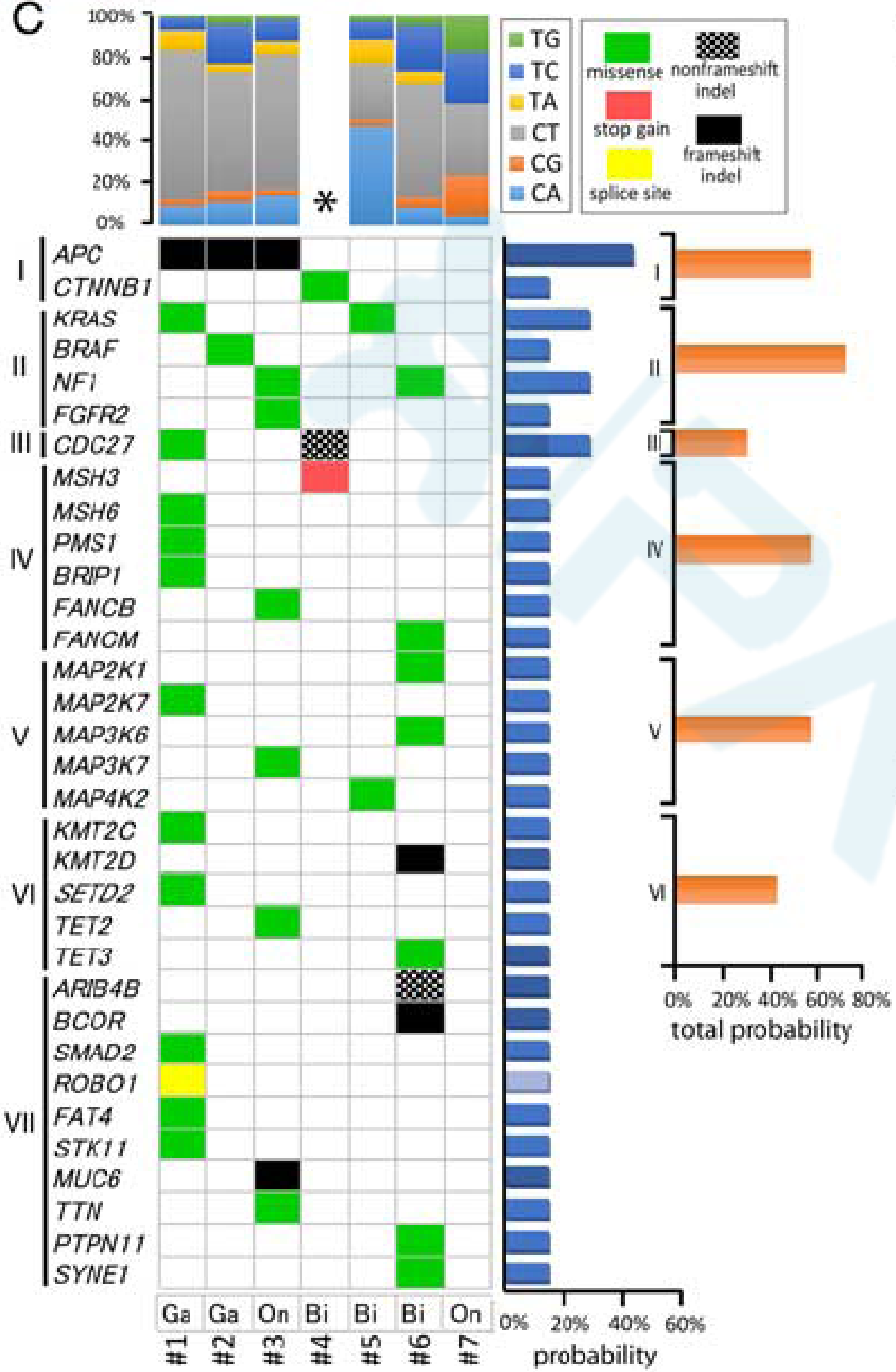
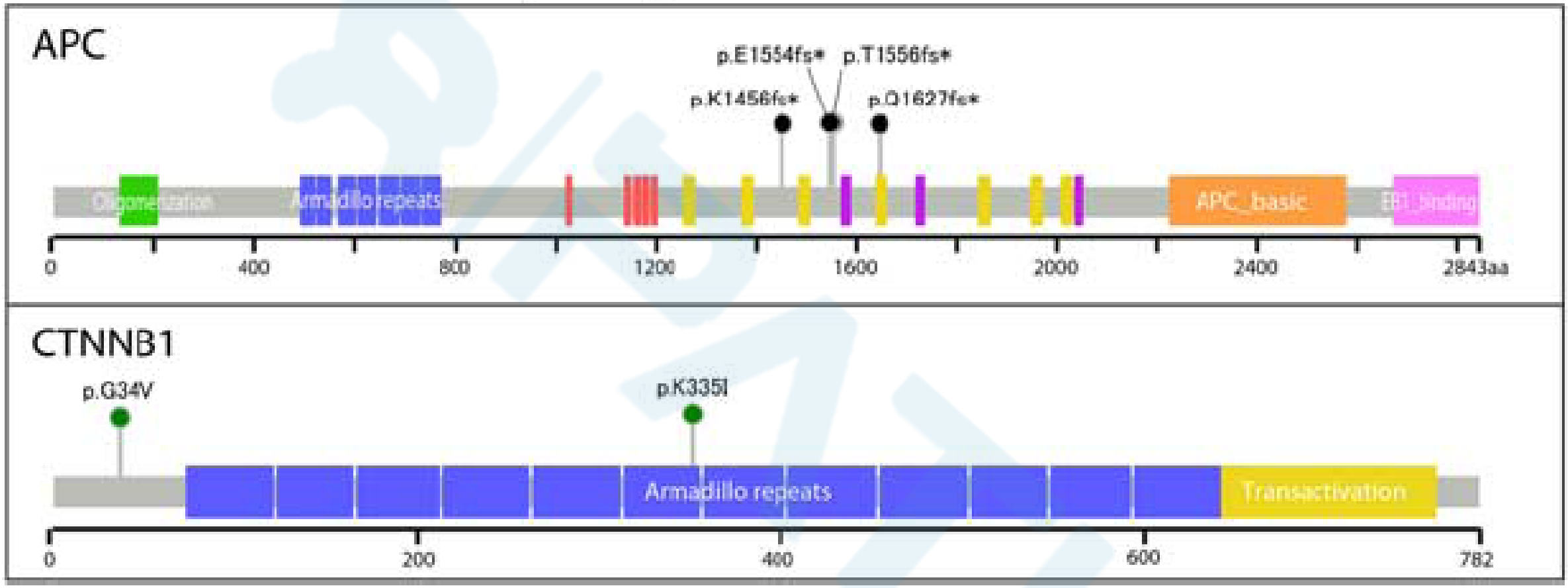


FIGURE 3. Identification of driver genes in biliary papillary neoplasms via exome sequencing. A, Tissue samples were dissected from papillary tumors in the dilated intrahepatic bile duct. B,Mutation spectra defined by the trinucleotide context. The height of each bar (the y axis) represents the proportion of somatic mutations that fall into a particular trinucleotide mutational context. Along the x axis, mutations are ordered in the alphabetical order of the reference trinucleotides (with themutated nucleotide in themiddle, from A[C>A]A to T[T>G]T) from the left to the right.



C,. Detection of somatic mutations The left lower plot shows information for genes with mutations in 7 cases. Mutations most likely related to the tumorigenesis are classified into the categories indicated on the left: (I) Wnt/β-catenin pathway; (II) RAS pathway; (III) cell cycle regulator; (IV) DNA repair gene; (V) MAP kinase; (VI) epigenetic modifiers; (VII) others. Green,missense; black, frameshift indel; black dot, nonframeshift indel; yellow, splice site. The upper histogram displays mutational signatures. Blue and orange histograms in the right indicate the percentages of genes and pathways affected (I to VI), respectively.

D



D, Positions of APC and CTNNB1 mutations found in biliary papillary neoplasms, and their relationship with protein domains. β-catenin binding repeats in APC are shown in pale red and orange boxes. Purple domains indicate the Axin binding domain.

RESULTS

- 4/7 cases (57%) had somatic mutations in genes involving the Wnt/β-catenin pathway
  - *APC* frameshift mutations in 3/7 cases (cases 1 to 3)
  - Case 4 had a somatic mutation in *CTNNB1*
- Driver mutations in bile duct cancers were also detected with *KRAS* or *BRAF* mutated in 3/7 cases
- Mutations in other genes involved in the RAS pathways (eg, *NF1* and *FGFR2*) were also identified

RESULTS

- CDC27 was mutated in 2 cases (29%)
  - A core subunit of the anaphase-promoting complex/cyclosome
  - Testicular germ cell tumors, prostate cancers and colorectal adenocarcinomas/adenomas
  - The specific roles remain unknown
- We also identified mutations
  - Epigenetic regulators (*KMT2C*, *KMT2D*, *TET2*, *TET3*, and *SETD2*)
  - DNA mismatch repair-related genes (*MSH3, MSH6, PMS1, BRIP1, FANCB,* and *FANCM*)
  - MAPK gene family genes (*MAP2K1, MAP2K7, MAP3K6, MAP3K7*, and *MAP4K2*)

RESULTS

- *TP53* is commonly mutated in biliary cancers, but were the wild-type in the 7 cases examined
- Gene mutations previously identified in IPNBs and pancreatic IPMNs (eg, *GNAS* and *RNF*) were not found

TABLE 2. *KRAS*, *GNAS*, *APC*, and *CTNNB1* Mutational Status in Biliary Neoplasms

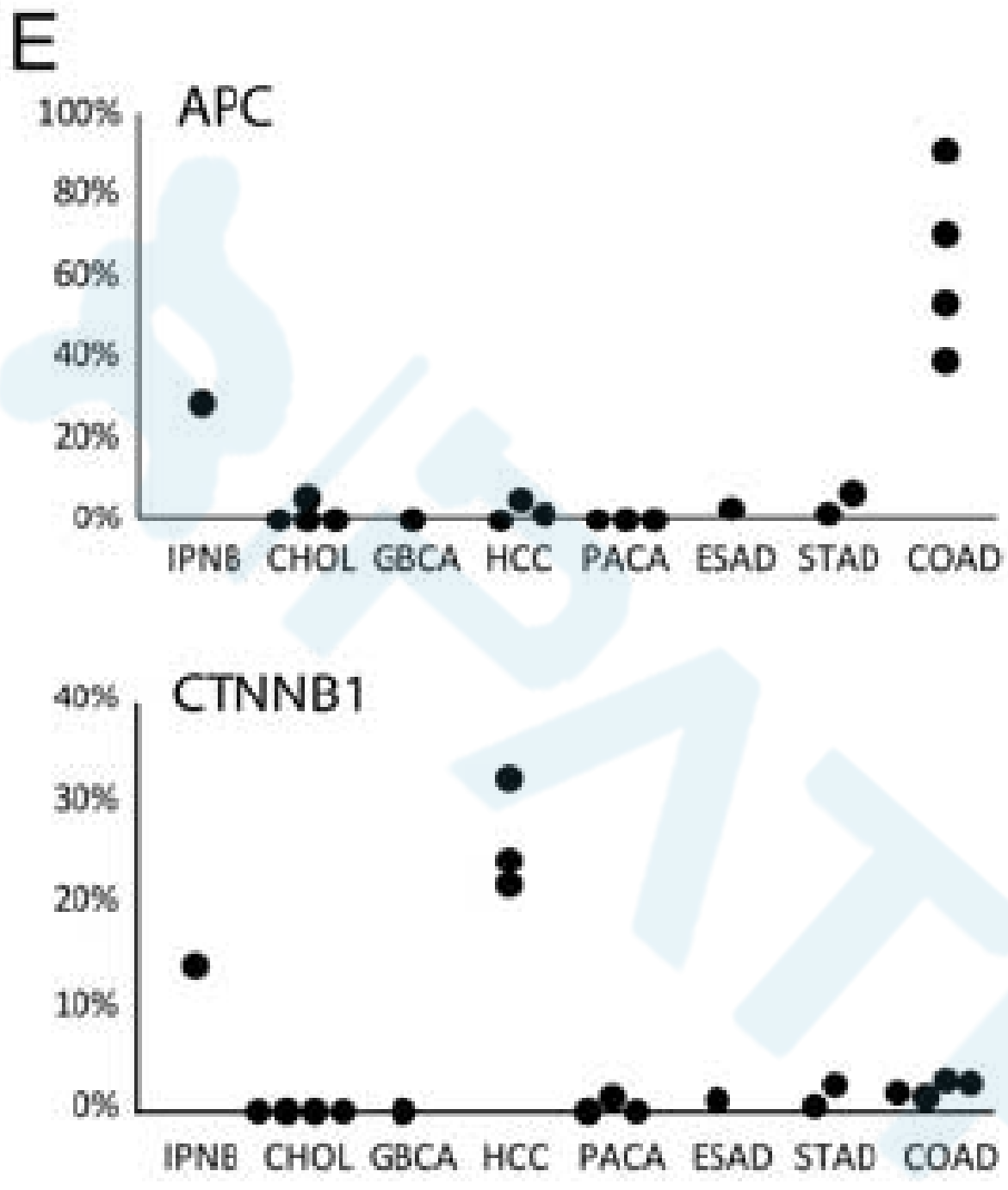
	Biliary Papillary Neoplasms (n [%])*			
	Total (N = 28)	IPNBs† (N = 14)	Papillary Cholangiocarcinomas (N = 14)	Nonpapillary Cholangiocarcinomas (N = 29) [(n [%])
KRAS	5 (18)	5 (36)	0‡	6 (21)
GNAS	0	0	0	0
APC	4 (14)	4 (28)	0	0**
CTNNB1	2 (7)	2 (14)	0	1 (3)
APC or *CTNNB1*	6 (21)	6 (43)	0‡	1 (3)**

*Meeting the current WHO definition of IPNBs.
†Fulfilling the stringent criteria of iIPNBs.[10]
‡$P < 0.05$ versus IPNBs.
**$P < 0.01$ versus IPNBs.

The frequency of *KRAS* mutations was also significantly higher in IPNBs than in papillary cholangiocarcinomas while GNAS mutations were not detected (36% vs. 0%; P = 0.041)
Mutations in *APC* and *CTNNB1* appeared to be almost restricted to IPNBs with 6/14 cases (43%)

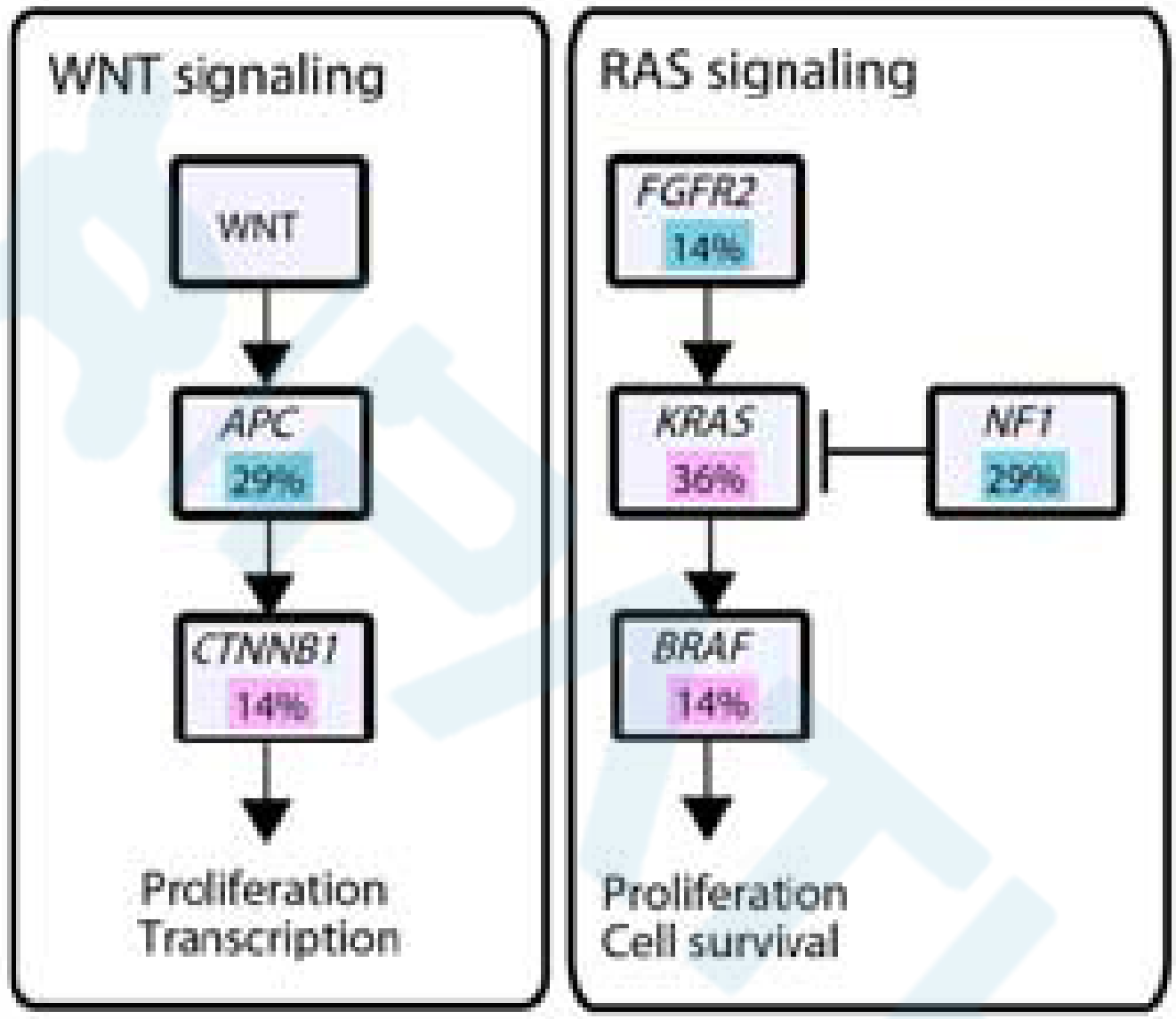
RESULTS

- The overall mutational profiles (eg, Wnt, RAS including *KRAS* and *BRAF, CDC27*, and DNA mismatch repair) of IPNBs differed from those of bile duct carcinomas
  - Genes involved in the Wnt/β-catenin signaling pathway appeared to be mutated at a significantly higher frequency in IPNBs than in cholangiocarcinomas (43% vs. 2%, $P < 0.001$)
  - DNA repair defects and *CDC27* truncating mutations identified in IPNBs are extremely uncommon in cholangiocarcinomas (0%, 0/98 cases according to the database)
- Wnt/β-catenin and RAS, were the major driver pathways in IPNBs with genes in these 2 pathways accounting for 10/14 cases (71%) in our cohort



E, Comparisons of the mutation frequencies of APC and CTNNB1 between IPNBs and various cancers (obtained from global cancer projects, see the Materials and methods section). CHOL indicates cholangiocarcinoma, 4 projects; GBCA, gallbladder carcinoma, 1 project; HCC, hepatocellular carcinoma, 3 projects; PACA, pancreatic carcinoma, 3 projects; ESAD, esophageal adenocarcinoma 1 project; STAD, stomach adenocarcinoma, 2 projects; COAD, colorectal adenocarcinoma, 4 projects.

F



F, Two major driver pathways in IPNBs: Wnt/β-catenin and RAS signaling. Alteration frequencies are expressed as a percentage in IPNBs. Red denotes activated genes and blue indicates inactivated genes.

TABLE 3. Clinicopathologic Summary and Genetic Features of IPNBs

No.*	Age (y)/ Sex	Location	Size (mm)	Subtype	Grade	Mutation Status (*APC*, *CTNNB1*, *KRAS*, and *BRAF*)	β-catenin Staining Cytoplasmic/ Nuclear)	Follow-up Period (mo)	Outcome
1	69/M	Hilar	16	Gastric type	High-grade dysplasia	*APC* p.E1554fs* *KRAS* p. G12D	2/0	15	No recurrence after surgery. Died of pancreatic cancer
2	65/M	Hilar	37	Gastric type	High-grade dysplasia with an associated invasive moderately differentiated adenocarcinoma of the pancreatobiliary type (pT2bN0M0)	*APC* p.Q1627fs* *BRAF* p. V600E, both found in noninvasive and invasive areas	3/0	5	No recurrence after surgery
3	75/F	Intrahepatic	22	Oncocytic type	High-grade dysplasia	*APC* p.K1456fs*	3/0	56	No recurrence after surgery
4	68/M	Intrahepatic	115	Pancreatobiliary type with an oncocytic component	High-grade dysplasia	*CTNNB1* p.K335I	3/0	60	No recurrence after surgery
5	78/M	Hilar	16	Pancreatobiliary type	High-grade dysplasia	*KRAS* p.G12V	0/0	16	No recurrence after surgery
6	30/F	Intrahepatic	110	Pancreatobiliary type	High-grade dysplasia with an associated invasive moderately differentiated adenocarcinoma of the pancreatobiliary type (pT3N1M1[ovary])	None in either noninvasive or invasive area	0/0	30	Died of the cancer derived from intraductal papillary biliary neoplasm
7	55/M	Hilar	45	Oncocytic type	High-grade dysplasia	None	3/0	60	No recurrence after surgery
8	68/M	Intrahepatic	25	Pancreatobiliary type	High-grade dysplasia with a small focus of mucinous carcinoma (pT1N0M0)	*KRAS* p.G12D	2/0	60	No recurrence after surgery
9	65/F	Distal	20	Gastric type	High-grade dysplasia with minor components of low-grade dysplasia	None in either low- or high-grade dysplasia	3/3	35	No recurrence after surgery
10	52/F	Hilar	87	Pancreatobiliary type	High-grade dysplasia	None	2/0	52	No recurrence after surgery
11	77/M	Intrahepatic	37	Pancreatobiliary type	High-grade dysplasia	*APC* p.T1556fs*	3/1	60	No recurrence after surgery
12	83/M	Intrahepatic	30	Oncocytic type	High-grade dysplasia	None	3/0	60	No recurrence after surgery
13	63/F	Intrahepatic	25	Gastric type with a pancreatobiliary component	High-grade dysplasia with minor components of low-grade dysplasia	*KRAS* p.G12S, *CTNNB1* p.G34V, same mutations in low- and high-grade dysplasia	0/0	25	No recurrence after surgery
14	79/F	Intrahepatic	10	Gastric type	High-grade dysplasia	*KRAS* p.Q61H	2/0	27	No recurrence after surgery

*Cases 1 to 7 were analyzed by whole exome sequencing, while case 8 to 14 underwent Sanger sequencing.
F indicates female; M, male.

The aberrant cytoplasmic and/or nuclear expression of β-catenin

- 5/6 IPNBs with *APC* or *CTNNB1* mutations
- 6/8 cases with wild-type *APC* and *CTNNB1* (total 79%)
- Papillary cholangiocarcinomas, all cases showed at least focal cytoplasmic expression

The expression values of β-catenin did not differ between IPNBs and papillary cholangiocarcinomas

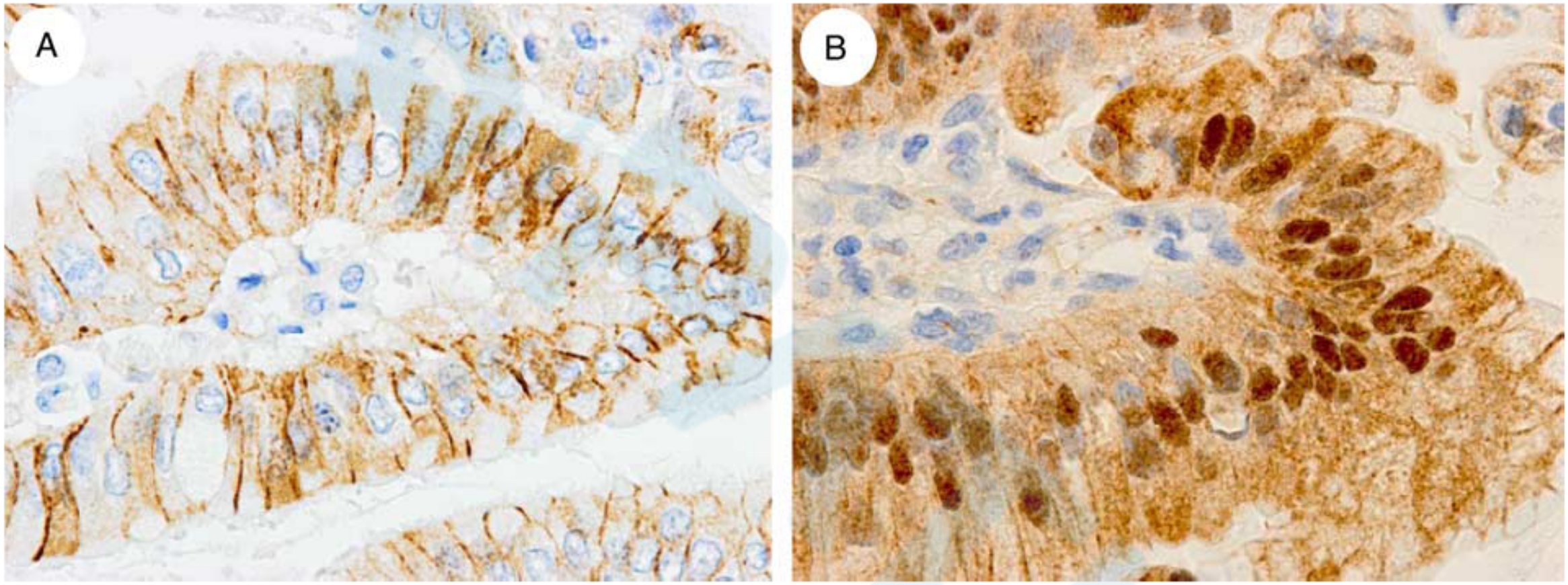


FIGURE 4. β-catenin immunohistochemistry in IPNBs. A, The mixed membranous and intracytoplasmic expression of β-catenin is observed (case 11). B, In addition to strong cytoplasmic expression, the nuclear accumulation of β-catenin is noted (case 9).

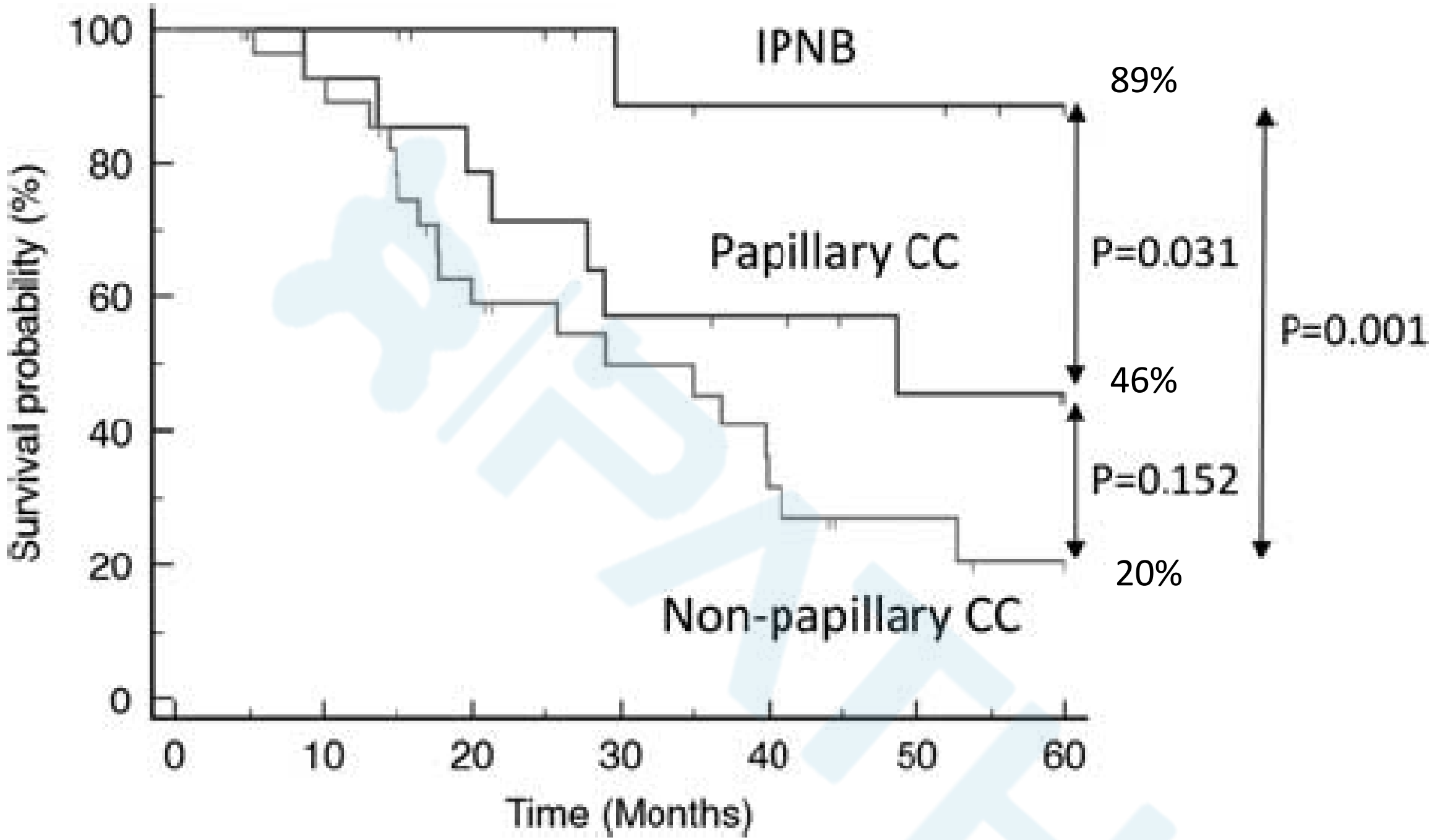


FIGURE 5. Overall survival analysis. Patients with IPNB show significantly better survival than those with papillary or non-papillary cholangiocarcinoma (CC).

DISCUSSION

- This global sequencing study on biliary papillary neoplasms revealed recurrent mutations in *APC* and *CTNNB1*
  - Mutations in these genes were restricted to IPNBs
  - Papillary cholangiocarcinomas lacked mutations in the Wnt/β-catenin signaling pathway
  - The better prognosis of patients with IPNB
  - Morphology-based separation scheme represents genetically distinct biliary papillary neoplasms
- Stage-matched prognostic comparison between the 2 groups will require future large-scale, multi-institutional studies

DISCUSSION

- Other regulatory mechanisms may play an important role in β-catenin activation
  - IPNBs with mutations in *APC* or *CTNNB1*
  - Without mutations also showed the cytoplasmic and/or nuclear expression of β-catenin
- IPNBs also appeared to be genetically distinct from the pancreatic counterpart
  - Mutations in *APC* and *CTNNB1* are observed in 0% and 6% of papillary pancreatic neoplasms, respectively
  - Gene mutations previously identified in IPNBs and pancreatic IPMNs (eg, *GNAS* and *RNF*) were not found

TABLE 4. Review of Previous Molecular Studies on IPNBs

		n (%)				
	Tumor Location	Wnt Pathway	*KRAS*	*GNAS*	*TP53*	Other Findings
Matthaei et al[14]	Intrahepatic (n = 6), extrahepatic (n = 13), both (n = 4), and gallbladder (n = 11)	Not analyzed	6/34 (18)	1/34 (3); an intrahepatic, intestinal-type case	Not analyzed	None
Schlitter et al[9]	Intrahepatic (n = 15), extrahepatic (n = 27), and both (n = 3)	Immunohistochemical nuclear expression of β-catenin in 4/45 (9)	16/45 (36)	1/44 (2); an intestinal-type case with low-grade dysplasia	Immunohistochemical nuclear expression of p53 in 30/45 (67)	*BRAF* 0/43 (0)
Sasaki et al[13]	Perihilar (n = 29) and distal (n = 6)	Not analyzed	12/26 (46)	15/30 (50); only 1 case (7%) was the intestinal type	Not analyzed	None
Tsai et al[12]*	Intrahepatic (n = 12), hilar (n = 8), both (n = 2), and distal (n = 19)	Not analyzed	13/41 (32)	12/41 (29); all cases were intestinal-type	Not analyzed	None
Tsai et al[39]*	Intrahepatic (n = 16), hilar (n = 15), and distal (n = 19)	Not analyzed	15/50 (30)	16/50 (32)	Not analyzed	*RNF43* 6/50 (12); 5 *RNF43*-mutated cases were intestinal-type; 4 had concurrent *GNAS* mutations
Present study	Intrahepatic (n = 8), hilar (n = 5), and distal (n = 1)	Mutations in *APC* or *CTNNB1* in 6/14 (43)	5/14 (36)	0/14 (0)	0/7 (0) (exome sequencing)	*BRAF* 1/17 (14), *CDC27* 2/7 (29), *RNF43* 0/7 (0)

*Study cohorts are overlapped.

CONCLUSION

- Activation of the Wnt/β-catenin signal pathway is relevant to the development and progression of this uncommon form of biliary papillary neoplasms
- IPNBs appeared to be not only morphologically, but also genetically distinct from papillary and nonpapillary cholangiocarcinomas
- Future functional studies are needed in order to clarify the biological consequences of *APC/CTNNB1* alterations in the biliary system

THANK YOU