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Original contribution

Reappraisal of pathological features of intraductal papillary neoplasm of bile duct with respect to the type 1 and 2 subclassifications☆

Yasuni Nakanuma MD, PhD [a,c,*], Katsuhiko Uesaka MD, PhD [b], Yukiyasu Okamura MD, PhD [b], Takuro Terada MD, PhD [d], Yuki Fukumura MD, PhD [e], Yuko Kakuda MD, PhD [a], Takashi Sugino MD, PhD [a], Yasunori Sato MD, PhD [f], Jang Kee Taek MD, PhD [g], Young Nyun Park MD, PhD [h]

[a] *Department of Diagnostic Pathology, Shizuoka Cancer Center, Shizuoka, 411-8777, Japan*
[b] *Department of Hepatobiliary Pancreatic Surgery, Shizuoka Cancer Center, Shizuoka, 411-8777, Japan*
[c] *Department of Diagnostic Pathology, Fukui Prefecture Saiseikai Hospital, Fukui, 918-8503, Japan*
[d] *Department of Hepatobiliary Pancreatic Surgery, Fukui Prefecture Saiseikai Hospital, Fukui, 918-8503, Japan*
[e] *Deparatment of Human Pathology, Juntendo University School of Medicine, Tokyo, 918-8503, Japan*
[f] *Department of Human Pathology, Kanazawa University School of Medicine, Kanazawa, 920-8640, Japan*
[g] *Department of Diagnostic Pathology, Samsung Medical Center, Seoul, 06351, South Korea*
[h] *Department of Diagnostic Pathology, Yonsei Medical Center, Seoul, 03722, South Korea*

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Summary The pathological spectrum of intraductal papillary neoplasm of bile duct (IPNB) remains to be clarified. A total of 186 IPNBs were pathologically examined using the type 1 and 2 subclassifications proposed by Japanese and Korean biliary pathologists incorporating a two-tiered grading system (low-grade and high-grade dysplasia), with reference to four subtypes (intestinal [i], gastric [g], pancreatobiliary [pb], and concocytic [o] subtype). IPNBs were classifiable into type 1 composed of low-grade dysplasia and 'high-grade dysplasia with regular structures' (69 IPNBs), and type 2 of 'high grade dysplasia with irregular structures and complicated lesions' (117 IPNBs). Type 1 was more common in the intrahepatic bile duct (78%), whereas type 2 was frequently located in the extrahepatic bile duct (58%). Mucin hypersecretion was more common in type 1 (61%) than in type 2 (37%). IPNBs were classifiable into the four subtypes: 86 iPNBs, 40 gIPNBs, 31 pbIPNBs, and 29 oIPNBs. The four subtypes were histologically evaluable with reference to the type 1 and 2 subclassifications. iIPNB and pbIPNBs were frequently classified as type 2, whereas types 1 and 2 were observed at similar rates in

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* Corresponding author. Department of Diagnostic Pathology, Fukui Prefecture Saiseikai Hospital, Wadanakacho Funahashi 7-1, Fukui, 918-8503, Japan
E-mail address: nakanumayasu@gmail.com (Y. Nakanuma).

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gIPNB and oIPNB. Stromal invasion was almost absent in type 1, irrespective of subtype, but was found in 66 of 117 type 2 IPNBs ($P < .01$), and postoperative outcome was favorable in IPNBs without invasion compared with IPNBs with invasion ($P < .05$). The type 1 and 2 subclassifications with reference to the four subtypes may provide useful information for understanding IPNB.

1. Introduction

Since intraductal papillary neoplasm of bile duct (IPNB) was proposed as a unique biliary tract neoplasm approximately 20 years ago, many studies have been reported, revealing that IPNB is a unique preinvasive, grossly visible neoplasm, frequently associated with invasive carcinoma, but that is not homogeneous in its clinicopathological features or molecular alterations [1−9].

Historically, IPNBs have been studied with reference to and comparison with intraductal papillary mucinous neoplasm of the pancreas (IPMN), because the biliary tract and pancreas are located in close anatomic proximity and some biliary diseases show similarities to pancreatic diseases [5,6,10−14]. Interestingly, the neoplastic lining epithelia of IPNB shows intestinal (iIPNB), gastric (gIPNB), pancreatobiliary (pbIPNB), and oncocytic (oIPNB) differentiation, as is observed in IPMN [1−3,14]. It became clear that IPNB shared several features with IPMN but also differed from IPMN in its higher histological grade, high frequency of associated invasive cancer, worse prognosis, and some differences in the oncogenic pathway [4−7,12−16]. Through these comparative studies, IPNB is now being established as an independent disease along the biliary tree [1,2,5,14].

Recently, a panel of Japanese and Korean biliary pathologists proposed the consensus that IPNBs are subclassifiable into types 1 and 2 by supplementing a traditional two-tiered grading system (low-grade and high-grade dysplasia [LGD and HGD]) [17]. Type 1 IPNB is characterized by regular structures, whereas type 2 shows irregular structures, and foci of complicated lesions, such as cribriform or solid structures, are frequently observed in type 2. Although type 1 IPNBs share histologic features with prototypic IPMNs, type 2 IPNBs are variably different from IPMNs [1,5,14,17].

The World Health Organization published the Classification of Digestive System Tumours 5th edition (2019), in which the term *IPNB* was defined and described as a unique preinvasive lesion of bile duct, and the type 1 and 2 subclassifications were only briefly and concisely introduced [1]. Along with this publication, several studies using the subclassifications have been published [8,15,18−20]. The type 1 and 2 subclassifications were reproducible in these studies. Most importantly, a multivariate analysis of factors associated with long-term postoperative outcomes showed that the subclassification was only significant factor and that type 1 was associated with a favorable postoperative outcome in comparison to type 2 [8,18]. In addition, type 1, particularly iIPNB, frequently showed *GNAS* and *KRAS* mutations, identical to those of IPMN, whereas type 2 did not [8,19,21]. However, the precise pathological evaluation of types 1 and 2, with reference to other pathological features (including four subtypes), one of the characteristics of IPNB, have not been discussed and remain to be clarified. In addition, although tubular structures are not infrequent in IPNBs, particularly gIPNB [1,15], the presence of tubular components has not been discussed in IPNB with respect to its diagnosis and subtyping. In fact, predominant tubular components in intraductal papillary tumors have been reported as an exclusion criterion of IPNB [22,23].

In this context, a pathological review of IPNB cases with respect to the type 1 and 2 subclassifications is merited. For this purpose, we collected 186 IPNBs, one of the largest series of pathological studies of IPNB yet reported. This study protocol was approved by the institutional review board of Fukui Saiseikai Hospital (2019−036) and of Shizuoka Cancer Center (T2020-71-2020-1-3).

2. Materials and methods

2.1. Pathologic definition of IPNB

IPNBs are defined as a grossly visible intraductal papillary biliary neoplasm covering fine fibrovascular stalks in dilated bile ducts and lacking an ovarian-type stroma [1,2]. This study included cases with histologically predominant papillary or villous lesions, as well as those with variable amounts of tubular structure; exclusion criteria of IPNB because of tubular-predominant features was not adopted [23,24]. IPNBs associated with invasive carcinomas were included, because invasion could develop as a natural course of IPNB and 'IPNB associated with invasive carcinoma' is evaluable and recognizable histologically [1,2].

2.2. Collection and preparation of tissue specimens

We collected a total of 180 cases with IPNB from many institutions from Japan, Korea, and Thailand (the hospitals from which these cases were obtained are shown in the Acknowledgment). The cases showing considerable amounts of two different subtype components within the

tumor like a collision tumor (n = 3), and the cases showed two different intraductal components of different subtypes at different anatomical levels of the bile duct (n = 3), were examined separately. However, if more than two intraductal tumors of the same subtype were found separately at different anatomical levels, they were examined as a single IPNB. When more than two subtypes were admixed in an intraductal tumor, the predominant subtype was examined. A total of 186 IPNB lesions from 180 cases were thus examined.

As for backgrounds of IPNB, Thailand cases (n = 34) had a history of eating raw fish, suspicion of a history of liver fluke infection by *Opisthorchis viverrini* (data cited from [25]). In Japanese cases (n = 135), a history of liver fluke infection was not obtained, and two cases were associated with hepatolithiasis. Both groups were compared in the proportion of four subtypes and of types 1 and 2 classification.

The number of tissue blocks including the main tumor of IPNB in individual cases ranged from 2 to 40. These blocks were fixed in 10% neutral-buffered formalin and embedded in paraffin. Data regarding the resected specimens and the main clinical and laboratory data were available. While the long term postoperative outcomes were available in 62 cases of Shizuoka Cancer Center, they were not available in the remaining cases.

More than 20 serial thin sections were cut from each formalin-fixed-paraffin-embedded-tissue block. Deparaffinized sections were stained routinely. The remaining sections were used for the immunohistochemical detection of MUC1, MUC2, MUC5AC, MUC6, CDX2, CK7, and CK20. Other antigens such as S100P were detected; however, the antibodies and other reagents and staining methods applied in individual hospitals were heterogeneous, and some immunostained sections were not available at the time of the present study. Thus, in this study, the immunostained sections were only referenced at the time of subtyping, and no comparative analyses were performed using these sections.

2.3. Pathological examinations of IPNBs

2.3.1. A two-tiered grading: LGD and HGD

Based on cytoarchitectural alterations, particularly nuclear changes, intraductal IPNB tumors were graded as LGD or HGD, as in other neoplastic precursor lesions in the pancreas and biliary tract [1,2,26].

LGD is a neoplastic lesion lacking cellular and nuclear pleomorphism, which shows mild nuclear hyperchromasia, and small or medium cells and nuclei (Fig. 1A). Nuclear stratification is not found, except in the case of iIPNB and gIPNB (foveolar area). Nucleoli are not evident, except in the case of oIPNB. The structures appear to be well-organized.

HGD is a neoplastic lesion showing cellular and nuclear enlargement and pleomorphism, nuclear hyperchromasia and stratification, a thickened nuclear membrane, prominent or evident nucleoli, and variable loss of cellular and nuclear polarity. The presence of any HGD area in the whole tumor indicates HGD [1,26]. Structures, such as papillary, villous, and tubular (glandular) growth, are relatively regular, and well-organized in some IPNBs, and they were categorized as 'HGD with regular structures', whereas in other IPNBs, their structures were irregular or heterogeneous, with lesions categorized as 'HGD with irregular structures' (Fig. 1B).

2.3.2. Type 1 and 2 subclassifications

Intraductal tumor is subclassified into types 1 and 2, according to the type 1 and 2 subclassifications [1,14,17].

Type 1: (1) "Papillary" or "villous" or "tubular" structural patterns are regular and appear homogenous and well-organized (Fig. 1A). (2) Fibrovascular stalks are thin, but not infrequently widened by edematous and inflammatory changes, particularly in the oncocytic subtype. (3) This type is composed of LGD and 'HGD with regular structures.'

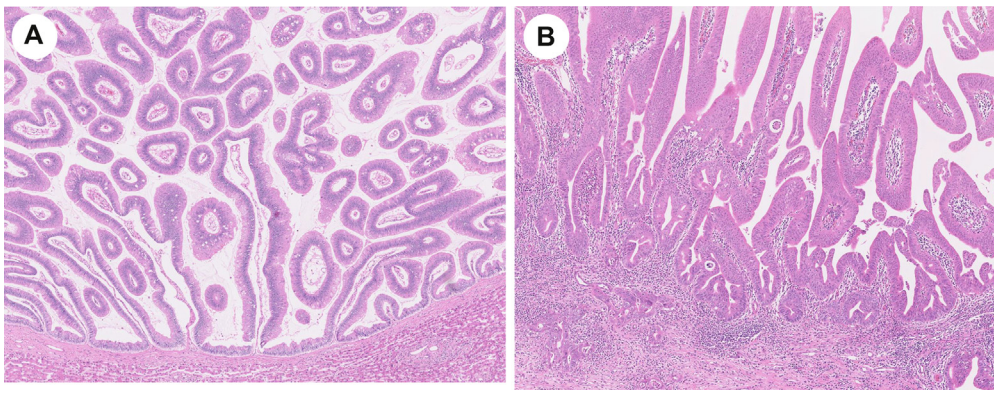

Fig. 1 The intestinal subtype of intraductal papillary neoplasm of bile duct (IPNB). A: Type 1. A well-organized villous pattern with fine fibrovascular stalks of low-grade dysplasia is growing regularly in the intrahepatic bile duct (HE staining x 70: original magnification). B: Type 2. Villous and papillary growth with intestinal epithelial morphology showing irregular structures and nuclear hyperchromasia, stratification and disordered polarity and stromal invasion of the duct wall (lower left). High-grade dysplasia. (HE staining x 70: original magnification).

Type 2: (1) "Papillary" or "villous" or "tubular" structural patterns are irregular and heterogeneous or not-well organized (Fig. 1B). A serrated epithelial lesion as seen in traditional serrated adenoma of the intestine [1] may be found. (2) Relatively thin, but variably thickened fibrovascular stalks often associated with irregular or complicated branching can be focally found. (3) Other lesions: (a) foci of complicated structures, such as cribriform, solid and compact tubular structures and variable-sized cystic changes, (b) foci of bizarre cellular and nuclear changes with the appearance of overt malignancy, (c) foci of other types of carcinoma, such as neuroendocrine carcinoma, and (d) coagulative necrosis can be detected. All cases of this type belonged to 'HGD with irregular structures.'

Type 1 is a prototype of each subtype of IPNB and resembles the prototypic subtype of IPMN and colorectal tubular, tubulovillous or villous adenoma. Type 2 can be regarded as variably deviated from the prototype (type 1) of IPNB.

2.3.3. Classification into four subtypes

A total of 186 IPNBs were classified into four subtypes based on the histological features and epithelial cell lineages [1,2,4,14]. Although their immunohistochemistry was helpful, there were overlapping immunohistochemical features in many cases.

2.3.3.1. Intestinal subtype (iIPNB). iIPNBs presented as papillary or villous or tubular neoplasms showing an intestinal epithelial differentiation. The lining epithelia were characterized by tall, columnar epithelia with single-layered or pseudostratified cigar-shaped enlarged nuclei and basophilic or amphophilic cytoplasm with varying apical vesicular mucin. The basic structures of iIPNB were of villous, papillary, or tubular patterns. Villous and papillary neoplastic epithelia covered the fine fibrovascular stalks, and tubular neoplastic epithelia were embedded in fibrous stroma. iIPNBs resembled the prototypic subtype of iIPMN, as well as colorectal tubular, or villous or tubulovillous adenoma. Immunohistochemically, CDX2 was expressed in almost all the cases examined, and CK20 was also frequently expressed. MUC2 was expressed in goblet cells.

2.3.3.2. Gastric subtype (gIPNB). gIPNB presented with regional growth of papillary and tubular (gladular) neoplastic epithelia resembling gastric foveola epithelia and pyloric glands, respectively. Immunohistochemically, gIPNBs were positive for CK7. Although foveolar components were positive for MUC5AC, and MUC6 could be detected in the pyloric gland portions, their expression was not exclusive, and both could be detected in the foveolar and pyloric portions simultaneously.

2.3.3.3. Pancreatobiliary subtype (pbIPNB). pbIPNBs showed many fine papillary structures with numerous fine ramifying branches of thin fibrovascular stalks, as seen in pbIPMN [1,2]. The structures and cellular patterns were characterized by two features: (1) many branching, fine papillary fibrovascular stalks with numerous ramifying branches (fern-like) and (2) single-layered, small- to medium-sized cuboidal or low-columnar, neoplastic-lining epithelial cells, usually with centrally or basally located, small- to medium-sized nuclei and pale or slightly acidophilic cytoplasm. The cellular and nuclear sizes resembled non-neoplastic biliary lining, simple epithelia. Immunohistochemically, the lining epithelia were frequently positive for CK7 and also for S100P and MUC1. MUC5AC was frequently expressed, but not constant. Staining of CDX2 and CK20 was negative.

2.3.3.4. Oncocytic subtype (oIPNB). oIPNBs were characterized by single- to multilayered medium-sized cuboidal to low-columnar epithelia with eosinophilic granular cytoplasm referencing prototypic oIPMN [1,2]. In the present study, oIPNBs showing small nuclei without prominent nucleoli were considered LGD, whereas those showing enlarged cells and hyperchromatic nuclei with prominent nucleoli were considered HGD. Immunohistochemically, CK7 and MUC5AC were frequently but variably expressed, and MUC6 was not infrequently expressed in the neoplastic epithelia.

2.3.4. The evaluation of other features of IPNBs

2.3.4.1. Anatomical location along the biliary tree. Extrahepatic IPNBs were located in the extrahepatic bileducts. Intrahepatic IPNBs were located within the liver parenchyma and the bile ducts proximal to the right or left hepatic ducts.

2.3.4.2. Mucin hypersecretion. .

2.3.4.3. Invasion. Colloid carcinoma, oncocytic carcinoma, and tubular adenocarcinoma were examined in the invasive areas.

2.4. Statistical analyses

Statistical significance was determined using Student's *t*-test, a chi-squared test, and Fisher's exact test. Estimates of overall survival were calculated using the Kaplan−Meier method using 62 cases of IPNB experienced in Shizuoka Cancer Center. That is, overall survival between IPNBs with invasion (33 cases) and without invasion (29 cases), between LGD (2 cases) and HGD (27 cases) without invasion, and

between type 1 (12 cases) and type 2 (17 cases) without invasion were compared using the long-rank test. *P* values of $<.05$ were considered to indicate statistical significance.

3. Results

3.1. Prevalence of type 1 and 2 subclassifications and of LGD and HGD

In 186 IPNBs, type 2 (63%) was common in comparison with type 1 (37%) (Table 1A). Most IPNBs were HGD (90%), whereas only 10% belonged to LGD (Table 1B). IPNBs of HGD (n = 167) was composed of 'HGD with regular structures' (30%) and 'HGD with irregular structures' (70%). Type 1 IPNB was composed of LGD (19 cases) and 'HGD with regular structures' (50 cases), whereas type 2 IPNB of only 'HGD with irregular histologies.'

3.2. Characterization of four subtypes with respect to the type 1 and 2 subclassifications

iIPNB was most common, followed by gIPNB, pbIPNB, and oIPNB (Table 1C).

Table 1A Main clinicopathological features of type 1 and 2 subclassifications of a total of 186 intraductal papillary neoplasm of bile duct (IPNB) lesions.

	Type 1	Type 2
Number of lesions (%)	69 (37%)	117 (63%)
Age (years): mean and range	66:46–82	67:34–90
Male:Female	42:27	81:36

%, percentage of lesions. All but one autopsy case were surgically resected cases. Two Japanese cases had a history of preceding hepatobiliary diseases (alcoholic fibrosis and chronic hepatitis C), whereas the cases from Korea and Thailand had a clinical history of eating habits suggestive liver fluke infestation [25]. There were no significant differences in the age or sex distribution between two subclassifications.

3.2.1. Histological characteristics of the four subtypes with respect to types 1 and 2

Histopathologies of four subtypes were evaluable based on type 1 and 2 subclassifications (a modified two-tiered grading system) as follows:

3.2.1.1. Intestinal IPNB (n = 86). In type 1 (n = 28), the structures of villous/papillary, tubular patterns were regular (Fig. 1A), whereas in type 2 (n = 58), they showed irregular, heterogeneous lesions with focally widened

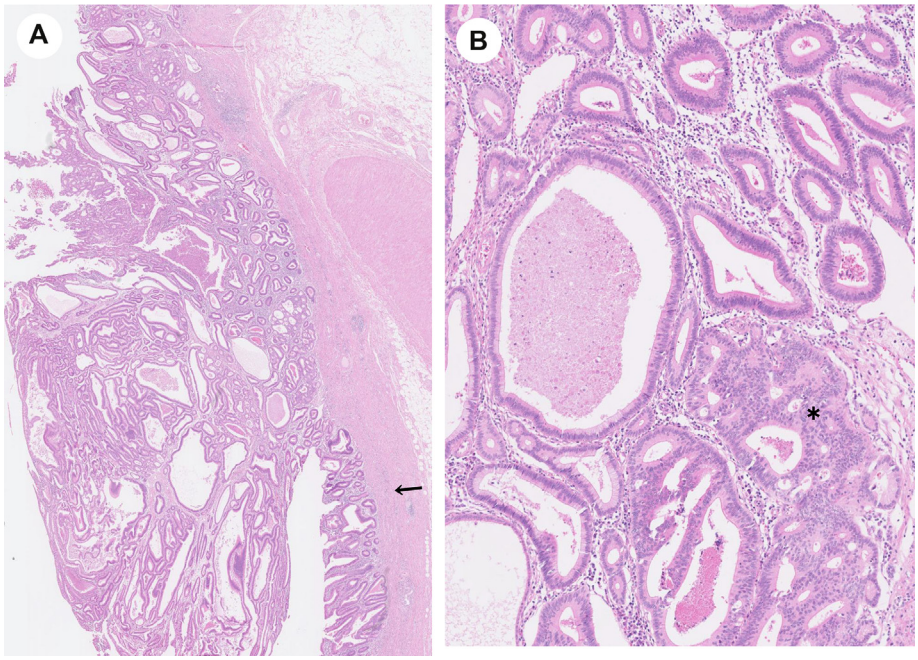

Fig. 2 The intestinal subtype of intraductal papillary neoplasm of bile duct (IPNB). Type 2. A: This tumor is mainly composed of an irregular tubular (glandular) pattern with thin stroma in the bile duct. The neoplastic tubules are pleomorphic in size, and some are cystic. The surrounding mucosa also shows an intraepithelial, noninvasive micropapillary neoplasm (→) (HE staining ×50: original magnification). B: A high-power view of "A" reveals nuclear hyperchromasia, stratification and disordered polarity of glandular epithelia, as well as complicated lesions, such as a cribriform pattern (*). High-grade dysplasia (HE staining ×150: original magnification).

Table 1B Correlation between distribution of low-grade dysplasia (LGD) and high-grade dysplasia (HGD) and that of type 1 and 2 subclassifications in a total of 186 intraductal papillary neoplasm of bile duct (IPNB) lesions.

	37%	100%
	Type 1 (69)	Type 2 (117)
LGD (19)	HGD (167)	
10%		100%

Type 1 IPNBs (n = 69) were composed of LGD lesions (n = 19) and of 'HGD lesions with regular structure' (n = 50), whereas type 2 IPNBs (n = 117) were composed of only 'HGD with irregular structures' (n = 117). This means that 50 of IPNBs with HGD (n = 167) were classified as type 1 ('HGD with regular structures') and the remaining 117 IPNBs were classified as type 2 ('HGD with irregular structures').

fibrovascular stalks or stroma (Figs. 1B, 2A and 2B). The nuclei were enlarged, along with their nucleoli, and the supranuclear vesicular mucinous appearance was decreased in type 2. Regarding the predominant structures, 27 IPNBs (all type 1) were mainly composed of villous/papillary pattern (more than two-thirds), whereas 10 IPNBs (1 type 1, n = 1; type 2, n = 9) had a mainly tubular pattern (more than two-thirds). The remaining 49 lesions (all type 2) were composed of mixed papillary/villous and tubular components (each more than one-third).

3.2.1.2. Gastric IPNB (n = 40). In type 1 (n = 19), the basic structures of the gIPNB showed a regional distribution of neoplastic areas resembling the gastric foveola (Fig. 3A) and those resembling pyloric glands (Fig. 3B) in variable proportions. The foveolar area was composed of columnar to low columnar cells with basally oriented, single-layered or pseudostratified nuclei and abundant supranuclear pale vesicular mucinous cytoplasm lining the thin fibrovascular stalks. The pyloric gland components were arranged as glandular structures embedded in a few fibrous stroma with cuboidal or low columnar epithelia, basally located nuclei and abundant clear supranuclear cytoplasm. In type 2 (n = 21), the two component patterns were irregular or immature. Recognition of the transition between the foveolar regions and pyloric gland region were not infrequently controversial (Fig. 3C). The lining epithelial cells of the foveolar components were composed of low to tall

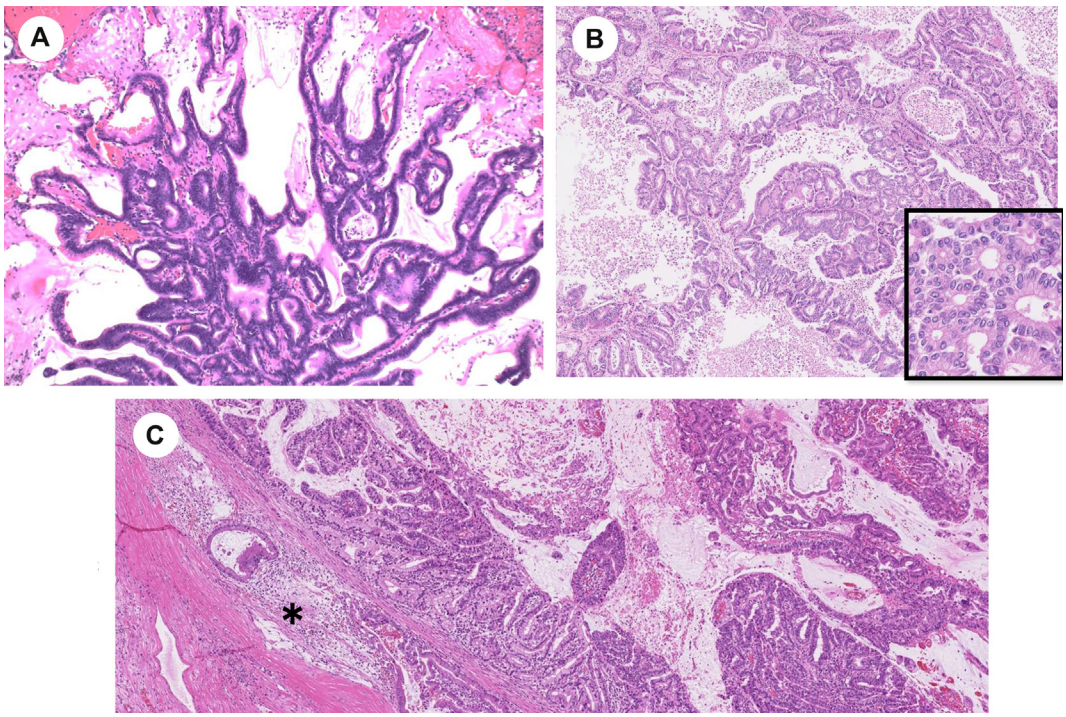

Fig. 3 The gastric subtype of intraductal papillary neoplasm of bile duct (IPNB). Type 1. A: Type 1. Papillary lesions with features resembling gastric foveola are seen in the bile duct with excessive mucus secretion (HE staining ×100; original magnification). B: Type 1. Beneath the papillary patterns of the surface, there are many glandular (tubular) components resembling gastric pyloric glands in the bile duct. Inset: Glandular structures are evident (HE staining ×100; original magnification). C: Type 2. Papillary lesions with tubular or acinar components in the bile duct. Foveolar and pyloric gland differentiation become vague, and stromal invasion was also found (*) (HE staining ×100: original magnification).

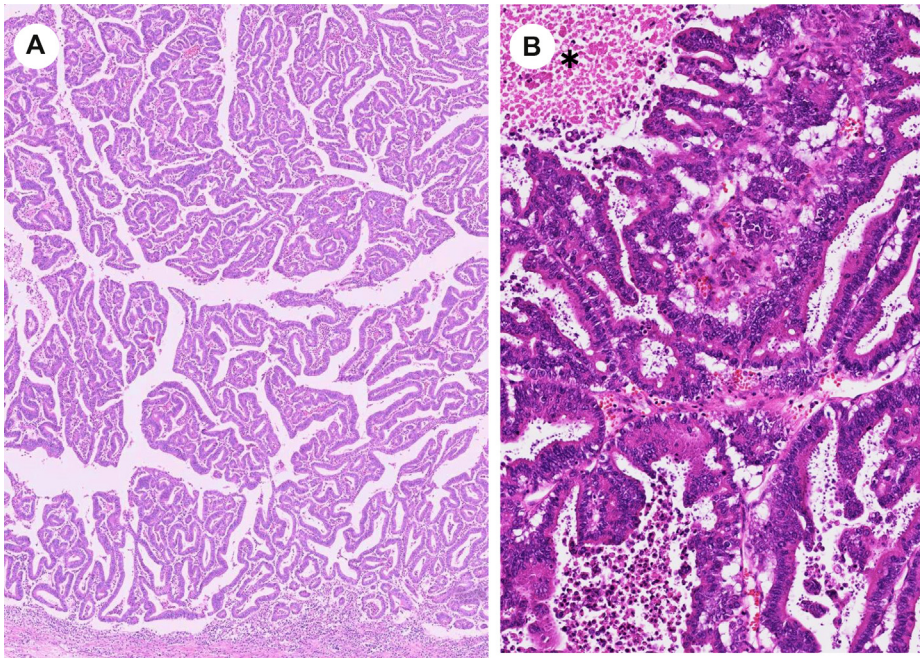

Fig. 4 The pancreatobiliary subtype of intraductal papillary neoplasm of bile duct (IPNB). A: Thin ramification of papillary branches lined by a single layer of epithelia. Inset: The lining epithelia with a single layer of nuclei resemble bile duct epithelia. Type 1, high-grade dysplasia (HE staining ×70: original magnification). B: Fine fibrovascular branches lined by atypical and papillary epithelia showing stratification and disordered polarity. Type 2, high-grade dysplasia (HE staining ×200: original magnification).

columnar epithelia with basally located, enlarged, hyperchromatic and anisocytotic nuclei and clear cytoplasm. Regarding the pyloric gland components, irregularly shaped papillary patterns with single-layered nuclei, or variably sized cystic changes with clear cytoplasm were also found. Structurally, 8 gIPNBs (type 1, n = 5; type 2, n = 3) were pyloric-gland-like pattern-predominant, 23 IPNBs (type 1, n = 8; type 2, n = 15) were foveola-like pattern-predominant, and the remaining 9 lesions (type 1, n = 4; type 2, n = 5) were mixed foveola-pyloric gland-like patterns.

3.2.1.3. Pancreatobiliary IPNB (n = 31). In type 1 (n = 6), many fine papillary structures with numerous fine

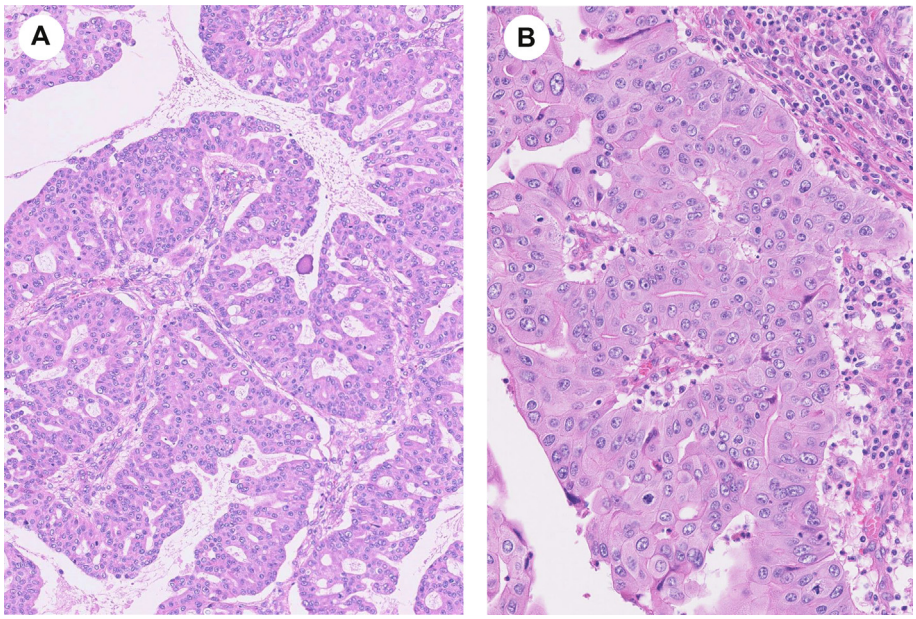

Fig. 5 The oncocytic subtype of intraductal papillary neoplasm of bile duct (IPNB). A: Compact growth composed of oncocytic epithelia with slit-like lumina. Type 1, low-grade dysplasia (HE staining ×150: original magnification). B: Compact growth of oncocytic epithelia with intratumoral lumina and disordered structures. Type 2, high-grade dysplasia (HE staining ×200: original magnification).

ramifying branches of thin fibrovascular stalks were homogeneous and regular throughout the tumor (Fig. 4A), whereas in type 2 (n = 25), papillary architectures with numerous irregular branching patterns, usually lined by single-layered or mild-stratified medium to large cells with hyperchromatic nuclei and prominent nucleoli, were irregular (Fig. 4B). Although fibrovascular stalks were usually fine, these stalks were occasionally widened at the basal side, and some areas were associated with suspected foci of stromal invasion of carcinoma.

3.2.1.4. Oncocytic IPNB (n = 29). In type 1 (n = 16), "papillary lesions" of single- to multilayered lining epithelia on fibrovascular stalk or "compact growth" of neoplastic epithelia with regular secondary lumina on thin fibrovascular or edematously widened stalks were found (Fig. 5A). The neoplastic lining epithelia of both patterns were medium-sized cells with relatively substantial, oncocytic and finely granular cytoplasm and small nuclei with small nucleoli. Foci of neoplastic epithelia of gastric subtypes were not infrequently found, particularly in "papillary lesions." Cytoplasmic hyalines were occasionally encountered in both patterns. Type 2 (n = 13) showed irregular "papillary lesions" and "compact" larger cells and nuclei with prominent nucleoli covering thin or edematous, inflammatory fibrovascular stroma. Fibrovascular stalks became irregular, and secondary lumina became irregular or ectatic and contained mucin (Fig. 5B).

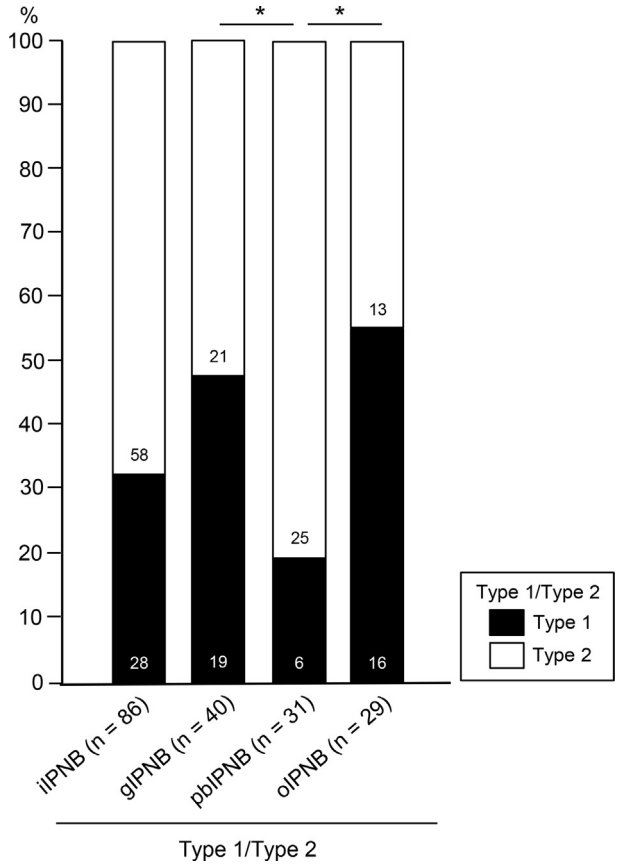

Fig. 6 The proportion of type 1 and 2 intraductal papillary neoplasms of bile duct (IPNBs) in four subtypes. In gastric IPNB (gIPNB) and oncocytic IPNB (oIPNB), the proportion of types 1 and 2 were similar (approximately 50%), whereas intestinal IPNB (iIPNB) and pancreatobiliary IPNB (pbIPNB) were more frequently classified as type 2 than as type 1. There were significant differences in the proportions of types 1 and 2 between pbIPNB and gIPNB or oIPNB. n: number of IPNB lesions. *, $P < .05$.

3.2.2. Relationship between type 1 and 2 subclassifications and the four subtypes

As shown in Fig. 6, in iIPNB and pbIPNB, type 2 (68% and 81%) was more frequently observed than type 1, whereas in gIPNB and oIPNB, types 1 (48% and 55%) and 2 (52% and 45%) were observed with similar frequency. Type 2 was significantly predominant in pbIPNB in comparison with gIPNB and oIPNB (both $P < .05$).

3.3. The prevalence of other features in IPNB with respect to the type 1 and 2 subclassifications

3.3.1. Location along the biliary tree

Overall, IPNBs were slightly frequent in the intrahepatic (55%) than in the extrahepatic bile duct (45%) (Fig. 7). As for the subclassifications, type 1 IPNBs were common in the intrahepatic (78%), whereas type 2 were frequent in the extrahepatic bile ducts (58%) ($P < .01$). As for four subtypes, iIPNBs and pbIPNBs were frequent in the extrahepatic, whereas gIPNB and oIPNB were frequent in the intrahepatic bile duct. There were significant differences in the intrahepatic and extrahepatic predominance between iIPNB and gIPNB or oIPNB, and between pbIPNB and gIPNB or oIPNB.

3.3.2. Mucus hypersecretion

Overall, 46% of 186 IPNBs showed mucus hypersecretion (Fig. 8). As for the type 1 and 2 subclassifications, 61% of the type 1 IPNBs (n = 69) showed mucus hypersecretion, whereas only 37% of the type 2 IPNBs (n = 117) showed mucus hypersecretion ($P < .01$). As for the four phenotypes, mucus hypersecretion was most common in gIPNBs (72.5%), followed by oIPNB (72.4%), iPNB (34%), and pbIPNB (19%). There were significant differences between gIPNB and iIPNB or pbIPNB and between oIPNB and iIPNB or pbIPNB.

3.3.3. Occurrence of complicated lesions, bizarre cells or nuclei, and coagulation necrosis

Foci of complicated lesions, bizarre cells or nuclei, and coagulative necrosis were only found in type 2 IPNBs (52%, 19%, and 29%, respectively; Fig. 9). Neuroendocrine carcinoma was only found in one case of type 2 IPNB.

3.3.4. Stromal invasion

Stromal invasion (Figs. 1B and 3C) was found in 38% of the 186 IPNBs (Fig. 10). As for the subclassification, type 2 showed frequent stromal invasion (56%), whereas type 1 showed invasion (6%) ($P < .01$). As for individual subtypes, stromal invasion was most frequent in pbIPNB (65%), followed by iIPNB (35%), oIPNB (28%), and gIPNB (25%). Such invasion was more frequent in pbIPNB than in either of the other subtypes ($P < .01$). The invasive parts of all pbIPNBs and gIPNB showed tubular carcinoma, while that of oIPNB showed oncocytic adenocarcinoma except for one case showing mixed colloid and tubular adenocarcinoma. While 27 iIPNBs showed invasive tubular adenocarcinoma, 2 iIPNBs mixed colloid and tubular adenocarcinoma, and one iIPNB predominantly colloid carcinoma.

3.3.5. Postoperative survival

As shown in Fig. 11, IPNBs without invasion showed favorable postoperative outcome in comparison with IPNBs with invasion ($P < .05$). However, there was no statistical difference in noninvasive IPNBs between LGD and HGD ($P > .05$) and between types 1 and 2 ($P > .05$).

3.3.6. Suspected backgrounds

There were no statistical significance in the proportion of four subtypes and type 1 and 2 subclassifications between Japan and Thailand cases (Table 1D).

4. Discussion

The findings obtained are summarized as follows: (1) based on the LGD and HGD status and structural regularities and complicated lesions, IPNBs were subclassified into types 1 (37% of all IPNBs) and 2 (63%). (2) Type 1 was common in the intrahepatic bile ducts and frequently showed mucin hypersecretion, whereas type 2 was frequent in the extrahepatic bile ducts and frequently showed stromal invasion. The type 1 and 2 subclassifications may provide useful information for understanding IPNB.

Although a two-tiered grading system (LGD and HGD) is used for the precursor lesions in the pancreatobiliary system [1,2,26], all or almost all IPNBs are classified as HGD or carcinoma in situ [8,27–29]. In the present study, 90% of IPNBs were classified as HGD and only the remaining 10% classified as LDG. This marked deviation of IPNB toward HGD and the possibility of sampling error

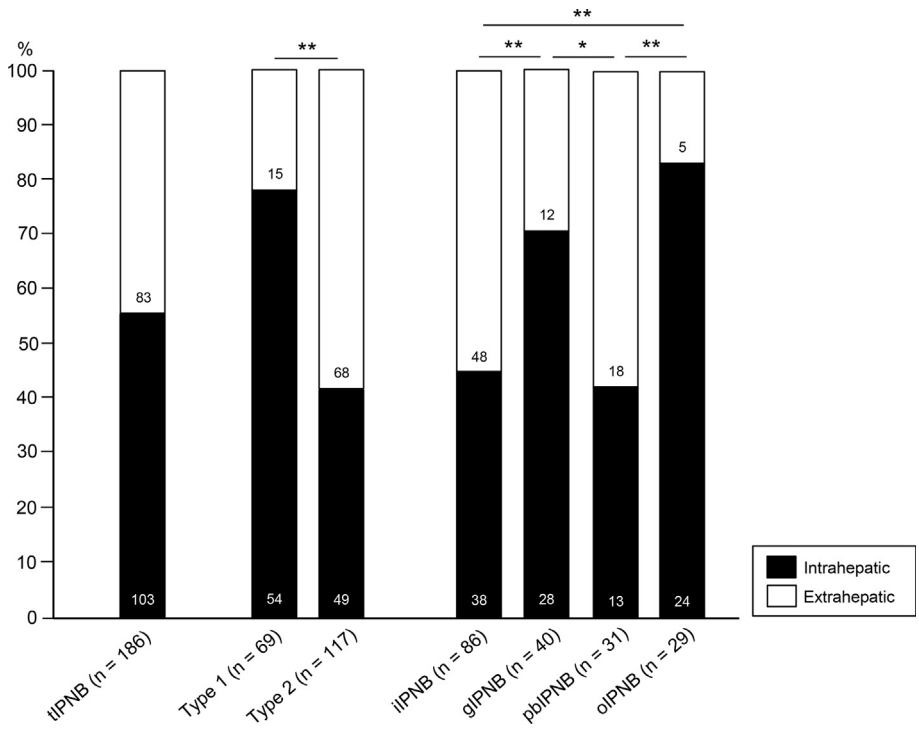

Fig. 7 The distribution of intraductal papillary neoplasms of bile duct (IPNBs) in the intrahepatic and extrahepatic bile duct. Total IPNBs (tIPNBs, n = 186), overall, were slightly frequently located in the intrahepatic bile duct (n = 103) than in the extrahepatic bile ducts (n = 83). Type 1 IPNBs were frequently located in the intrahepatic bile duct, whereas type 2 IPNBs were frequent in the extrahepatic bile ducts ($P < .01$). Intestinal IPNB (iIPNB) and pancreatobiliary IPNB (pbIPNB) were similarly distributed between both bile ducts, whereas gastric IPNB (gIPNB) and oncocytic IPNB (oIPNB) were preferentially located in the intrahepatic bile ducts. There were significant differences between gIPNB and iIPNB or pbIPNB, and between oIPNB and pbIPNB or iIPNB. n, number of IPNB. *, $P < .05$; **, $P < .01$.

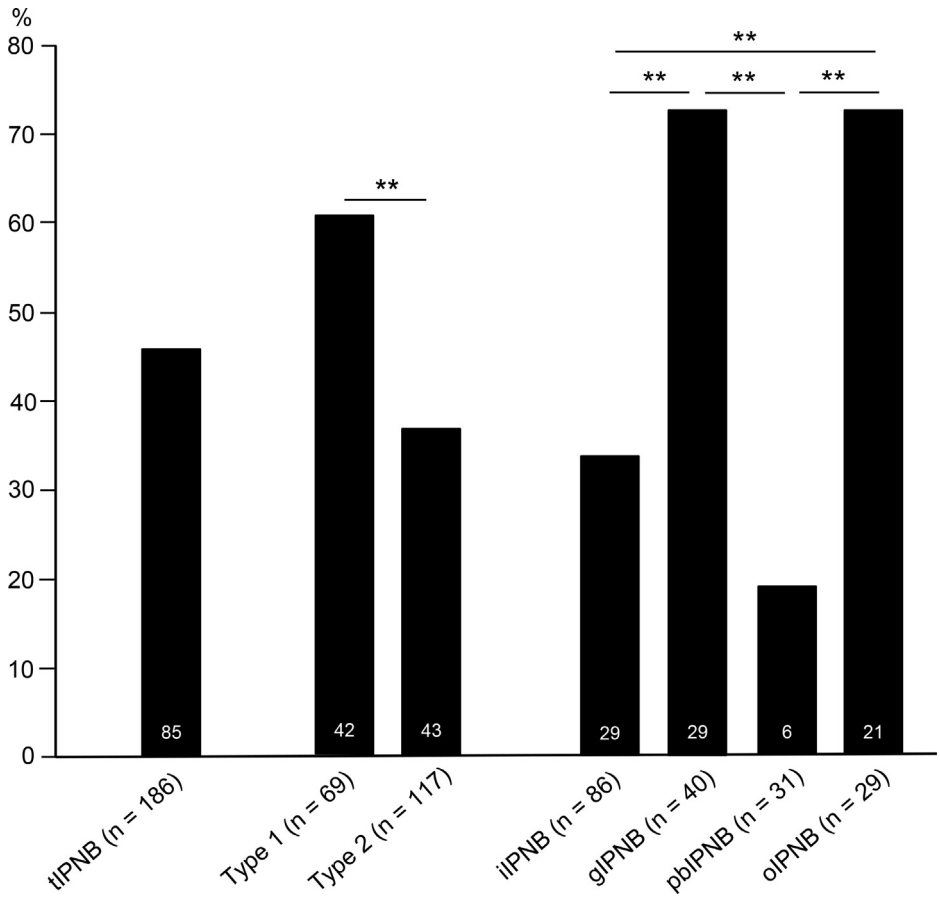

Fig. 8 The incidence of mucin hypersecretion in intraductal papillary neoplasms of bile duct (IPNBs). About half of total IPNBs (tIPNBs, n = 186) showed mucin hypersecretion. As for the type 1 and 2 subclassifications, type 1 IPNB showed mucin hypersecretion more frequently than type 2 IPNBs ($P < .01$). Gastric IPNB (gIPNB) showed mucin hypersecretion most frequently, followed by oncocytic IPNB (oIPNB), intestinal IPNB (iIPNB), and pancreatobiliary IPNB (pbIPNB). There were significant differences between gIPNB and iIPNB or pbIPNB, and between oIPNB and pbIPNB or iIPNB. n, number of IPNBs. **, $P < 0.01$.

suggest the supplementation of a two-tiered system to grade IPNBs. This study showed that the type 1 and 2 subclassifications [1,17] were actually applicable in a large number of IPNBs and could be supplementary. In fact, this subclassification system was reproducible in several studies [8,15,18–20].

Through this review of the largest number of IPNBs, iIPNB was found to be the most common subtype, followed by gIPNB, pbIPNB, and oIPNB, and the four subtypes were then examined with reference to the type 1 and 2 subclassifications.

iIPNBs structurally showed (1) papillary/villous predominant, (2) tubular-predominant, and (3) mixed papillary/villous and tubular type. Villous/papillary predominant-iIPNB resembled iIPMN and colorectal villous adenoma. In contrast, tubular-predominant iIPNB, which is not recognized in iIPMN [1,2], resembled colorectal tubular adenoma. In high-grade colorectal tubular neoplasms, "papillary structures" develop structurally, along with tubular components [1]: mixed villous/papillary and tubular-type iIPNB may reflect or recapitulate features of high-grade colorectal tubular adenoma. Serrated IPNB lesions also mimicked traditional serrated adenoma of the intestine [1].

Our recent study [15] showed that two components suggestive of gastric foveola (eg, papillary epithelia) and

Table 1C Main clinicopathological features of intraductal papillary neoplasm of bile duct (IPNB) in a total IPNBs and in four subtypes of IPNB, respectively.

	tIPNB	iIPNB	gIPNB	pbIPNB	oIPNB
Number of IPNBs	186	86 (46.2%)	40 (21.5%)	31 (16.7%)	29 (15.6%)
Age (years): mean and range	66: 34–90	65: 40–80	66: 42–85	68: 40–90	64: 46–86
Male:Female (%)	122:64	60:26	27:13	19:12	16:13

A total of 186 IPNBs were subtyped into four subtypes. tIPNB, total IPNB; iIPNB, intestinal subtype of IPNB; gIPNB, gastric subtype of IPNB; pbIPNB, pancreatobiliary subtype of IPNB; oIPNB, oncocytic subtype of IPNB. There were no significant differences in the age or sex distribution among four subtypes.

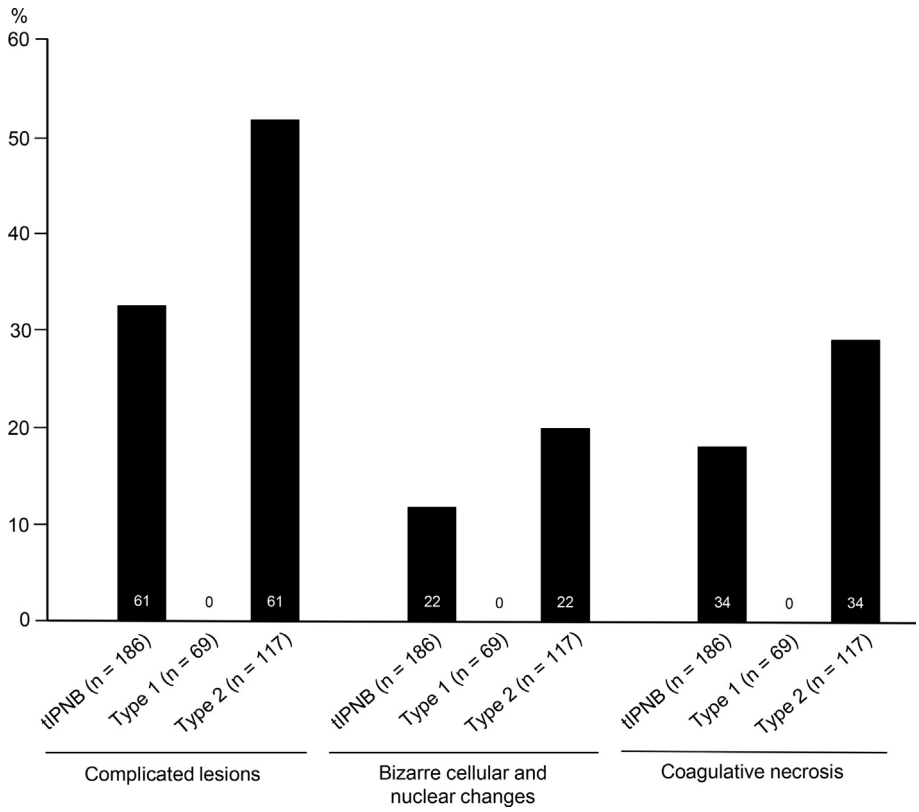

Fig. 9 The incidence of complicated lesions (cribriform and solid lesions, and variable-sized cystic lesions), bizarre cellular and nuclear changes, and coagulation necrosis in intraductal papillary neoplasms of bile duct (IPNB). These three categories of lesions were found in 61, 22, and 34 IPNB lesions, respectively, in tIPNBs (n = 186). These lesions were only detected in type 2 cases but not in type 1 cases. n, number of cases.

pyloric glands (eg, tubular epithelia) were found regionally in gIPNB, and the transition of both components was recognizable at several points. The present study showed that the pyloric gland-predominant type was most common, followed by the foveola-predominant type and mixed pyloric gland-foveola type. The pyloric gland-predominant gIPNB and gIPMN may correspond to previously reported intraductal tubular neoplasms or pyloric gland adenoma of the pancreatic ducts and pyloric gland adenoma or tubular adenoma of the bile duct [22,30,31].

There were at least two defining characteristics of pdIPNB: (1) many fine papillary structures and numerous ramifying branches with thin fibrovascular stalks and (2) a simple cuboidal and low-columnar epithelium with centrally or basally located nuclei resembling the lining epithelia of the bile duct. In this series, all pbIPNBs were classified as HGD. The differentiation of type 2 pbIPNB from other subtypes of type 2 was controversial in some cases because the cytological features of pancreatobiliary differentiation were not characteristic in comparison with other subtypes.

oIPMNs have been reported to present cast-like growth in the pancreatic ducts and rarely show mucus hypersecretion [1,2], and their genetic changes also differ from those of other subtypes of IPMN; thus, oIPMN was recently recognized as a distinct entity from the other three subtypes of IPMN [1]. However, oIPNB frequently presented with excessive mucin secretion in this study, suggesting that a difference in the biological features of oIPMN and oIPNB.

Recently, mucin- and MUC5AC-negative intraductal tubulopapillary neoplasms (ITPNs) and MUC5AC-positive intraductal tubule-forming biliary neoplasm were reported [22,23]. In those cases, tubule predominance was a criterion used to exclude a diagnosis of IPNB. However, this study suggested that tubule (gland)-predominant and mixed tubular-villous IPNBs, in addition to papillary/villous-predominant IPNBs, may be a part of the spectrum of gIPNB as well as of iIPNB. In this context, MUC5AC-positive biliary intraductal papillary neoplasm and other intraductal tubular neoplasms showing gastric and intestinal differentiations could be included in the spectrum of gIPNB and iIPNB.

Regarding the distribution along the biliary tree, IPNBs overall were slightly frequent in the intrahepatic bile ducts. Type 1 was frequent in the intrahepatic, whereas type 2 was frequent in the extrahepatic bile ducts. iIPNB and pbIPNB were frequently found in the extrahepatic bile ducts, whereas gIPNB and oIPNB were frequent in the

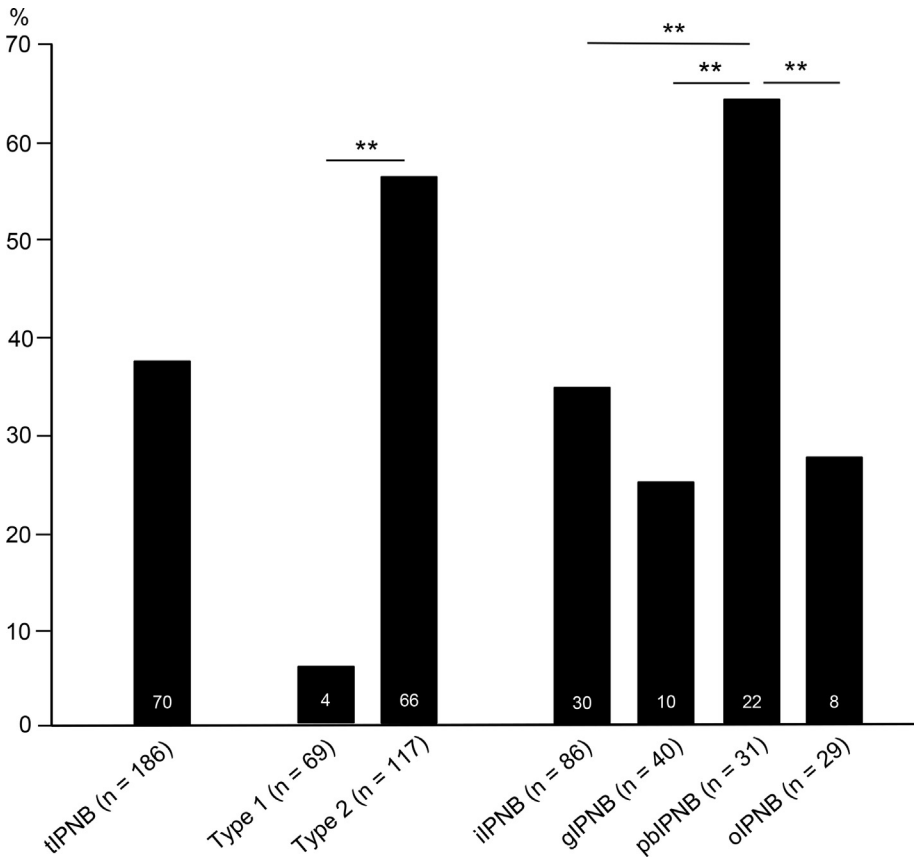

Fig. 10 The incidence of stromal invasion by intraductal papillary neoplasms of bile duct (IPNBs). Stromal invasion was found in 70 of the 186 in total IPNBs (tIPNBs). As for the type 1 and 2 subclassifications, 56% of the type 2 IPNBs showed stromal invasion, whereas only 6% of the type 1 IPNBs showed invasion ($P < .01$). Pancreatobiliary IPNB (pbIPNB) showed stromal invasion most frequently (22 of 31 cases), followed by intestinal IPNB (iIPNB), oncocytic IPNB (oIPNB), and gastric IPNB (gIPNB). There were significant differences between pbIPNB and oIPNB, gIPNB or iIPNB. **, $P < .01$.

intrahepatic bile ducts. The four subtypes IPMNs are also reported to show preferential anatomical locations [1,2]: gIPMNs are almost always found in the branch pancreatic duct, whereas iIPMNs are frequent in the main pancreatic duct. The anatomy may influence the development of IPNB with the four subtypes, as well as the type 1 and 2 subclassifications, as is observed in IPMN.

Table 1D Proportion of subtypes and type 1 and 2 subclassifications in Japan and Thailand.

	Japan (n = 135)	Thailand (n = 34)
Subtypes		
Intestinal (n = 75)	58 (43%)	17 (50%)
Gastric (n = 37)	30 (22.2%)	7 (21%)
Pancreatobiliary (n = 29)	26 (19.3)	3 (8.8%)
Oncocytic (n = 28)	21 (15.6%)	7 (21%)
Type 1 and 2 subclassifications		
Type 1 (n = 51)	37 (27.4%)	14 (41.2%)
Type 2 (n = 118)	98 (72.6%)	20 (58.8%)

This study demonstrated that mucin hypersecretion was common in type 1 but uncommon in type 2, and stromal invasion was frequent in type 2, but rare in type 1. Mucin hypersecretion is known to be more common in intrahepatic IPNBs than in extrahepatic IPNBs [4,7,32], and was relatively common in gIPNB and oIPNB. Stromal invasion, which is reported to be more common in the extrahepatic IPNB than in the intrahepatic IPNB, was relatively frequent in pbIPNB, which was prevalent in the extrahepatic bile duct. Taken together, the anatomic location of types 1 and 2 IPNB may influence several phenotypes of IPNB and reflect a postoperative prognosis in comparison to type 1 [8,18].

Thailand cases with a history of eating low fish, possible indicative of *O. viverrini* infection [25] and Japanese cases with no history of liver fluke but few occasional hepatolithiasis showed similar distribution of type 1 and 2 subclassifications and of four subtypes, suggesting that these background factors may not to be related to the development of these features of IPNB.

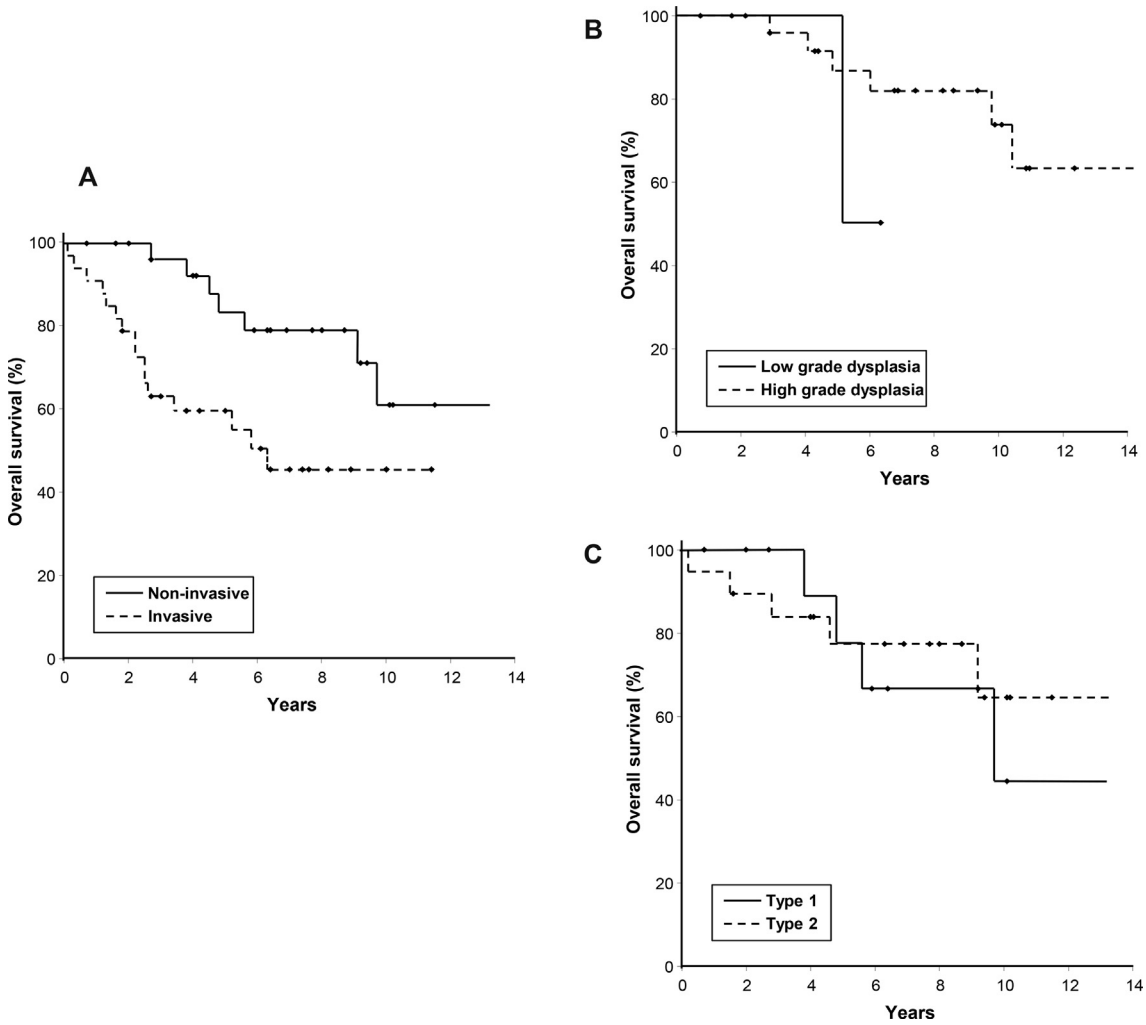

Fig. 11 Kaplan-Meier survival curves for cohorts of patients with (33 cases) and without invasion (29 cases), for non-inasive IPNBs with low-grade (2 cases) and high-grade dysplasia (27 cases) and for non-invasive IPNBs with type 1 (12 cases) and type 2 (17 cases). A: IPNBs without invasion showed a favorable outcome than those with invasion ($P = .0172$). B: There was no difference in noninvasive IPNBs between low-grade and high-grade dysplasia ($P = .343$). C: As for type 1 and 2 IPNBs without invasion, there was no significant difference ($P = .761$), while type 1 seems to show a favorable course until 5 years.

IPNBs and IPMNs share several features [10,14]. Mucin hypersecretion is common in both IPNBs and IPMNs, whereas it is not constantly in IPNBs [1,6,10,24]. In addition, almost all cases of gIPMN are classified as LGD [1,2], whereas 20% and 25% of gIPNBs were classified as HGD and showed stromal invasion, and iIPNBs were composed of villous/papillary-predominant, tubular (glandular)-predominant and mixed villous/papillary mixed growth patterns, similar to those in colorectal adenomas, whereas iIPMN only showed villous differentiation [1,2]. Most iIPMNs show *GNAS* and *KRAS* mutations [1,2]. However, although iIPNBs with a villous pattern showed genetic features similar to those of iIPMNs, other iIPNBs showed other genetic alterations, such as *TP53* and *PIK3CA* mutations [21]. Furthermore, in IPMNs, colloid carcinoma was a relatively common finding in areas of invasion [1,2], although this lesion was rare in IPNBs. Molecular and genetic studies targeting the differences between IPNB and IPMN may provide important information on the development of IPNB and also IPMN [10].

As for the factors related to postoperative course of IPNBs, Gordon-Weeks et al. reported a significantly worse prognosis for IPNB with invasion [33], and this was reproduced in this study. Recent several studies analyzing the postoperative course of IPNBs using many cases [8,20,29] revealed that type 1 showed favorable postoperative outcomes in comparison with type 2, suggesting that this subclassification may be applicable in the clinical evaluation of IPNBs. However, in this study, the significant better prognosis was not obtained in type 1, probably because the number of cases examined was too small. Several molecular and genetic studies on type 1 and 2 IPNBs [8,9,19–29,34] showed *KRAS* and *GNAS* mutations enriched in type 1, whereas *TP53*, *SMAD4*, and *KMT2C* mutations enriched in the type 2 [8,19].

The main limitations of this study were that the IPNB cases that were included in the present study included both hospital cases and consultation cases, raising the possibility of bias in this study. The results of molecular and genetic analyses were not available in this study. The subtyping of IPNB could be informative but detailed immunohistochemistries were limited in routine practice.

5. Conclusions

IPNBs were classifiable into the type 1 and 2 subclassifications based on structural regularities and the occurrence of complicated lesions. Types 1 and 2 show different clinicopathological features. The former frequently showed mucin hypersecretion and rarely showed stromal invasion, whereas that latter infrequently showed mucus hypersecretion and frequently showed stromal invasion and unfavorable postoperative outcome. Examination of invasion and two-tiered grading supplemented by type 1 and 2 subclassifications may give important information, and the recognition of type 1 and 2 IPNBs may improve the understanding of IPNBs.

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