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**Authors:** Sciarra A, Missiaglia E, Trimech M, Melloul E, Brouland JP, Sempoux C, La Rosa S

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# Gallbladder mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN) arising in intracholecystic papillary neoplasm. Clinico-pathologic and molecular analysis of a case and review of the literature

Amedeo Sciarra[1] (ORCID: 0000-0002-7550-0312), Edoardo Missiaglia[1], Mounir Trimech[1], Emmanuel Melloul[2], Jean-Philippe Brouland[1], Christine Sempoux[1], Stefano La Rosa[1] (ORCID: 0000-0003-1941-2403)

[1]Institute of Pathology, Lausanne University Hospital and University of Lausanne, 25 rue du Bugnon, 1011 Lausanne, Switzerland

[2]Department of Visceral Surgery, Lausanne University Hospital, 46 rue du Bugnon, 1011 Lausanne, Switzerland

**Corresponding author:**

Stefano La Rosa, MD
Institut Universitaire de Pathologie, CHUV
25 rue du Bugnon
CH-1011 Lausanne
Switzerland
Tel: +41 (0)21 3147162
Fax: +41 (0)21 3147205
e-mail: stefano.larosa@chuv.ch

**Abstract**

Mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) of the gallbladder are generally composed of adenocarcinoma and neuroendocrine carcinoma (NEC). Rare cases associated with intracholecystic papillary neoplasm (ICPN) have been reported. Although recent molecular data suggest that the different components of digestive MiNENs originate from a common precursor stem cell, this aspect has been poorly investigated in gallbladder MiNENs. We describe the clinico-pathologic and molecular features of a MiNEN composed of ICPN, adenocarcinoma and NEC. A 66-year-old woman presented with severe abdominal pain. She underwent radical cholecystectomy and an intracholecystic mass was found. Histologically, it was composed of ICPN associated with adenocarcinoma and large cell neuroendocrine carcinoma (LCNEC). The three components were positive for DNA repair proteins and p53. EMA was positive in the ICPN and adenocarcinoma components, while it was negative in the LCNEC. Heterogeneous expression of Muc5AC, cytokeratin 20, and CDX2 was only observed in the ICPN component. Cytokeratin 7 was diffusely positive in both adenocarcinoma and LCNEC components, while it was heterogeneously expressed in the ICPN. The copy number variation analysis showed overlapping results between the adenocarcinoma and LCNEC components with some minor differences with the ICPN component. The three tumor components showed the same mutation profile including *TP53* mutation c.700T>C (p. Tyr234His), without mutations in other 51 genes known to be frequently altered in cancer pathogenesis and growth. This finding may support the hypothesis of a monoclonal origin of the different tumor components. We have also performed a review of the literature on gallbladder MiNENs.

**Keywords:** gallbladder; MiNEN; MANEC; mixed neuroendocrine-non-neuroendocrine neoplasm

**Introduction**

Mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) are epithelial neoplasms constituted of a neuroendocrine and a non-neuroendocrine component. By definition, a neoplasm can be qualified as MiNEN when each component is morphologically and immunohistochemically recognizable and constitutes ≥30% of the tumor burden [1]. Rather as a single entity, the term MiNEN is considered as a conceptual category of neoplasms; indeed, different types of MiNEN can arise in different locations throughout the digestive system, and each of them shows peculiar clinico-pathological features related to the nature of the MiNEN components [1, 2]. The incidence of gallbladder MiNENs is not known, but recent data have suggested that more than one third of diagnosed gallbladder neuroendocrine carcinomas (NECs) are MiNENs because they are associated with a non-neuroendocrine component [3], which, in most of cases, is represented by an adenocarcinoma with different degree of differentiation [4]. To our knowledge, two cases of MiNEN in which the neuroendocrine (NEC) and non-neuroendocrine (adenocarcinoma) components were associated with intracholecystic papillary neoplasm (ICPN) have also been reported [5, 6].

Recent data showed that the different components of digestive MiNENs present the same molecular background suggesting that they originate from a common precursor stem cell [7-9]. However, among all the MiNENs described in these studies, only one was located in the gallbladder [8]. More recently, the molecular profile supporting the monoclonal origin of the two components has been demonstrated in another gallbladder MiNEN, composed of adenocarcinoma and NEC [10]. However, a molecular analysis of gallbladder MiNEN composed of ICPN, adenocarcinoma and NEC has never been performed. In the present study, we describe the clinico-pathological features of such a rare gallbladder MiNEN,

including the NGS mutational profile of the three tumor components. In addition, we performed a review of the literature on gallbladder MiNENs to give the reader a comprehensive overview on this rare tumor type.

**Case report**

A 66-year-old woman, known for occasional biliary colic during the last 2 years, presented with severe abdominal pain. Ultrasonography, CT scan and MRI showed a distended gallbladder containing a contrast-enhanced 9.5 cm sized mass, without dilatation of the intrahepatic bile ducts (Fig. 1A). Radical cholecystectomy with hepatic resection of the vesicular bed was performed. In addition, retro-duodenal lymph nodes as well as lymph nodes located in correspondence of the hepatic artery were resected.

Macroscopically, the lesion presented a friable, soft-tan, and exophytic growth (Fig 1B). Histologically, it mainly showed a tubulo-papillary architecture, composed of medium to large cells, with abundant eosinophilic cytoplasm and oval nucleus, corresponding to a papillary intracholecystic neoplasia (ICPN). Transition from low-grade to high-grade dysplasia was evident in some areas. A second tumor component representing about 30% of the tumor burden was identified and it was composed of atypical cells showing a glandular and cribiform architecture infiltrating the lamina propria without reaching the muscle. This second component had the histologic features of an adenocarcinoma. A third component was also identified and represented about 30% of the tumor burden. It showed a solid pattern of growth and was composed of large cells with vesicular nuclei and abundant eosinophilic cytoplasm (Fig. 1C and Fig. 2).

Immunohistochemistry (Fig. 2) was performed on a Ventana Benchmark XT autostainer (Ventana Medical System, Tucson, AZ, USA) using the antibodies listed in Table 1. Tumor cells of the three components showed preserved expression of DNA repair proteins hMLH-1, hMSH-2, hMSH-6, hPMS-2 and were positive for p53 (Fig. 3). EMA was positive in the first (ICPN) and second (adenocarcinoma) component, while it was negative in the third solid component. Heterogeneous expression of Muc5AC, cytokeratin 20, and CDX2 was only observed in the ICPN component, while it was negative in the other two. Cytokeratin 7 was diffusely positive in both adenocarcinoma and solid components, while it was heterogeneously expressed in the ICPN component. Tumor cells of the third solid component were positive for synaptophysin and chromogranin A: synaptophysin was positive in almost all cells, while chromogranin A in about 30% of cells. The adenocarcinoma component was negative for both neuroendocrine markers excluding the diagnosis of either amphicrine carcinoma or poorly differentiated adenocarcinoma with divergent neuroendocrine differentiation. Only rare scattered neuroendocrine cells were observed in the ICPN component. The Ki67 proliferation index of the LCNEC component was 50% and it was higher than the Ki67 index observed in the other two components (Fig. 4). Following the WHO guidelines, for the calculation of the Ki67 proliferative index all the immunohistochemically labeled nuclei, regardless of their staining intensity, were manually counted using the camera-captured, printed image (CCPI) technique. The number of Ki67 positive nuclei were counted in a total 1000 neoplastic cells and was then expressed as a percentage (“index”) of immunoreactive cells. Muc2 was negative in the three components. No lympho-vascular or perineural invasion was observed. Peritumoral gallbladder showed mild chronic cholecystitis and hepatic parenchyma was normal. The final diagnosis was

gallbladder MiNEN composed of ICPN, adenocarcinoma and LCNEC with no lymph node metastases (TNM stage: pT1a pN0).

The three MiNENs components (ICPN, adenocarcinoma, and LCNEC) were manually microdissected and subjected to targeted next-generation sequencing (NGS). An amplicon-based DNA library was prepared using a customized primer panel targeting hotspot regions of 52 cancer genes including *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53,* and *VHL* (custom Ion AmpliSeq panel, Ion Torrent, Thermo Fisher Scientific, Waltham, MA, USA), and subsequently sequenced on an Ion S5 System (Ion Torrent, Thermo Fisher Scientific, Waltham, MA, USA). The three tumor components showed the same *TP53* mutation c.700T>C (p. Tyr234His) (Fig. 3). All the other analyzed genes were wild type. The copy number variation analysis (CNV), performed from the read coverage, showed overlapping results between the adenocarcinoma and LCNEC components, including deletions of chromosomes 3, 5q, 12, and 18q. The ICPN component shared with the adenocarcinoma and LCNEC component the deletion of chromosomes 5q, 12, and 18q, but did not show chromosome 3 deletion. Conversely, deletion of chromosome 10q was observed only in the ICPN area.

We have also performed a revision of the literature; the PubMed database of the National Center for Biotechnology Information (NCBI) of the US National Library of Medicine was searched using the following string: gallbladder [AND/OR] mixed adenoneuroendocrine carcinoma [AND/OR] mixed neuroendocrine-non-neuroendocrine neoplasm. All articles written in English were included. In addition, since a heterogeneous terminology has been

used during the last years to define mixed neuroendocrine neoplasms [2], we revised the references lists of each paper selected in the PubMed database, with the aim to reduce the risk of missing cases not appropriately defined. For each identified article, the reported cases were singularly identified and the following parameters were considered: sex, age, clinical presentation, presence of gallstone, presence of local extension, presence of lymph node and distant metastases, morphology of neuroendocrine component, morphology of non-neuroendocrine component, treatment, and follow-up data. When considering the 44 gallbladder MiNENs reported in the literature, including the present case (Table 2) [4-6, 10-43], the median age at the diagnosis is 65 years (range: 34-85 years), with a predominance in female patients (M:F ratio = 0.3). The most common clinical presentations are non-specific gastrointestinal symptoms, leading to imaging work-up and incidental discovery of an intracholecystic mass. Gallstones are reported in up to 40% of the cases. At the time of diagnosis, 65% of tumors are limited to the gallbladder wall, with involvement of the serosal surface in 21% of the cases. Infiltration of adjacent organs is reported in 33% of patients. Of note, 51% of cases present liver, peritoneal, nodal or, more rarely, distant metastases at the time of diagnosis. The presence of these features significantly affect the prognosis. Indeed, 83% of patients with non-metastatic tumors limited to the gallbladder wall are alive and free of disease after a mean follow-up time of 12 months (range 0.33-45 months), compared to 22% for those with worrisome features (mean follow-up time of 6 months, range 3-44 months). It should be stressed that for gallbladder MiNENs, such as for other MiNENs located elsewhere, the histology and burden of the different components are factors likely affecting the prognosis [2].

**Discussion**

Gallbladder MiNEN is a rare neoplasm. In a recent series, this entity represented about 10% of gallbladder carcinomas and about 2% of all hepatobiliary carcinomas [4]. However, despite the limited number of reported cases, recent published data suggest that gallbladder MiNENs are probably more frequent than expected, because more than a third of diagnosed gallbladder NECs are associated with a non-neuroendocrine component, which, in most of cases, is represented by an adenocarcinoma [3]. Accordingly, most reported cases presented an adenocarcinoma component, with different degree of differentiation, associated with a neuroendocrine component most frequently represented by small cell carcinoma (35%). Rarely, additional components such as squamous cell carcinoma, sarcomatoid or osteosarcomatous differentiation have been described [13, 33, 34, 40, 42]. In two cases, the NEC and adenocarcinoma components arose in ICPN [5, 6] as the case described in the present report. By definition, MiNENs are neoplasms in which each malignant component should represent at least 30% of the tumor burden, although this cut-off has been arbitrarily chosen [1]. Based on this definition, mixed neoplasms in which one of the two components does not reach 30% should not be considered MiNENs. This aspect opens a crucial point of discussion because a focal (<30%) tumor component, especially if biologically aggressive like a NEC, can have a clinical impact influencing prognosis. Our suggestion is to describe in the pathology report each tumor component, especially if represented by a NEC, although it represents less than 30% of the tumor mass and, for this reason, the tumor cannot been defined MiNEN. In the present case, we observed the two malignant components (adenocarcinoma and LCNEC) associated with pre-neoplastic lesion (ICPN). The association of adenocarcinoma and NEC with a pre-neoplastic component is not a rare event in the digestive system as described in gastric or intestinal MiNENs in which the two malignant components (adenocarcinoma and NEC) coexists with an adenoma [2]. From

the review of the literature we found a gallbladder neoplasm presenting an association of NEC and ICPN [44]. However, by definition, this association should not be considered as a MiNEN, since ICPN is a non-invasive neoplastic proliferation [45,46]. According to the diagnostic criteria proposed in the last WHO classification of digestive neuroendocrine neoplasms, NENs associated with a non-neuroendocrine component consisting solely of a precursor (pre-invasive) neoplasm are not considered MiNENs [1]. The present case can be diagnosed as MiNEN because in addition of the ICPN component there are two malignant components.

Recent molecular analysis suggest that the different components of digestive MiNENs derive from the same precursor progenitor cell since they show an overlapping molecular background [7-9]. However, among all MiNENs investigated only two were located in the gallbladder [8, 10] and, for this reason, the molecular background of gallbladder MiNEN needs to be better clarified. In the present study, we performed a morphological, immunohistochemical and molecular analysis of a gallbladder MiNEN composed of ICPN, adenocarcinoma and LCNEC. Using an NGS approach, targeting 52 among the most frequently altered genes involved in cancer pathogenesis and growth, we evaluated the mutational profile of the three tumor components. Although we observed some minor differences in CNV features between LCNEC and adenocarcinoma components versus the ICPN one, the three components shared the same mutation of *TP53,* without mutations in other genes. This observation suggests that the three components have the same molecular background. This corroborates the hypothesis of a common origin of the different MiNEN components. Our molecular results are in line with those recently reported by Ines et al. who detected an exclusive *TP53* mutation in both the adenocarcinoma and NEC components of a

gallbladder MiNEN [10]. Noteworthy, *TP53* mutations is a rare event in ICPN, which more frequently shows *KRAS* mutations [46].

The molecular profile of neoplasms that correspond to the different components of our case (ICPN, adenocarcinoma and LCNEC) is well known. LCNECs frequently bear mutation of *TP53* and *RB1* [47]. However, in a recent study, point mutations in *RB1* gene were not detected in colorectal MiNENs, which were more frequently *BRAF*, and less frequently *KRAS* and *APC* mutated than adenocarcinomas [9]. The few available data on molecular profile of ICPN suggest that *KRAS*, *STK11*, *CTNNB1*, and *APC* are the major driver genes, as opposed to *TP53* mutation, which is the most frequent mutation found in papillary adenocarcinomas of the gallbladder [48]. Our findings suggest that the genotype of the present case does not perfectly fit with the typical genotype of respective neoplasms corresponding to the three MiNEN components. Indeed, we did not find the gene mutations frequently observed in ICPN and gallbladder adenocarcinomas, when they present in their pure form. The observation that the genotype of MiNEN components is not identical to their pure counterparts is in line with findings reported in a MiNEN of the lung composed of adenocarcinoma and NET (atypical carcinoid) where mutations in *KRAS* (Gly13Asp), *PAPPA2* (Arg901Leu) and *NF1* (Val2106Phe) genes where found [49]. *KRAS* mutation is extremely rare in pure lung carcinoid [50] as well as *PAPPA2* and *NF1* mutation in lung adenocarcinoma [51]. Similarly, in a recent study on digestive neoplasms composed of adenoma and NET [52], the two components did not show *KRAS* mutation, a frequent finding in colorectal adenomas [53], but nuclear accumulation of β-catenin, which is a very rare event in pure gastrointestinal NETs [54]. Taken together, all these findings may support the hypothesis that the different components of MiNENs are monoclonal, but their molecular signature is not identical to that of the relative counterparts when they present as separate neoplasms.

The molecular profile of MiNEN components seems to show a “hybrid” pattern including the presence or absence of specific gene alterations that are not usually observed in their corresponding neoplasms when presenting as pure forms.

The present study is the first report describing the clinico-pathological features of a gallbladder MiNEN composed of ICPN, adenocarcinoma and NEC also including a large molecular analysis of the three components and it gives new insights into the molecular signature of this rare cancer. Although our findings do not give an immediate clinical impact on the management of patients, it represents a start point of molecular data collection that can be useful for a future change of the therapeutic approach.

**Author contributions:** AS, SLR study design, data collection, drafting. CS, JPB: data collection. MT, EdM molecular analysis. EmM surgery. All authors approved the manuscript.

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**Compliance with Ethical Standards:**

**Conflict of Interest:** The authors declare that they have no conflict of interest

**Informed consent:** Informed consent was obtained from the patient

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**Figure legend**

**Figure 1.** Intraoperative ultrasound imaging showed a distended gallbladder containing a variably hypo-isoechoic mass (arrow) (A). At gross examination, the gallbladder contained a large polypoid lesion, protruding and obliterating the gallbladder lumen (B). Microscopically, the neoplasm showed three different tumor components: intracholecystic papillary neoplasms (ICPN, bottom left), adenocarcinoma with glandular and cribriform pattern (upper right), intimately admixed with a large cell neuroendocrine carcinoma (asterisk) showing a solid architecture (C).

**Figure 2.** Immunohistochemical profile of the three different MiNEN components. HE: hematoxylin-eosin, CK: cytokeratin.

**Figure 3.** Illustration of the *TP53* gene alteration and p53 immunohistochemical expression in the three tumor components. First row, next generation sequencing data, visualized with the Integrative Genomic Viewer (IGV) software, revealed an identical *TP53* missense mutation c.700T>C (p. Tyr234His) in the three components (forward and reverse reads are shown in red and blue, respectively). Second row, immunohistochemical staining with anti-p53 antibody revealed a similar intense and diffuse aberrant protein expression in the three components. ICPN: intracholecystic papillary neoplasm; NEC: neuroendocrine carcinoma.

**Figure 4.** Ki67 immunoreactivity in the three different tumor components. The Ki67 index is high in the LCNEC component and lower in the other two components. The field corresponds to that showed in figure 1C.

**Table 1.** Antibodies and antisera used.

| Antibody | Dilution | P/M (Clone) | Source |
|---|---|---|---|
| Synaptophysin | 1:100 | M (snp88) | BioGenex Laboratories, San Ramon, CA, USA |
| Chromogranin A | 1:1 | M (LK2H10) | Ventana Medical System, Tucson, AZ, USA |
| EMA | 1:100 | M (E29) | Dako, Carpinteria, CA, USA |
| MUC2 | 1:50 | M (CCP58) | Dako |
| MUC5A | 1:400 | M (CLH2) | Dako |
| CDX2 | 1:40 | M (CDX2-88) | BioGenex Laboratories |
| Cytokeratin 7 | 1:200 | M (OV-TL 12/30) | Dako, |
| Cytokeratin 20 | 1:80 | M (IT-Ks 20.8) | Progen Biotechnik, Heidelberg, Germany |
| p53 | 1:500 | M (DO-7) | Dako |
| hMLH1 | 1:1 | M (M1) | Ventana Medical System |
| hMSH2 | 1:1 | M (G219-1129) | Ventana Medical System |
| hMSH6 | 1:1 | M (44) | Ventana Medical System |
| hPMS2 | 1:1 | M (EPR3947) | Ventana Medical System |
| Ki67 | 1:100 | M (MIB1) | Dako |

P/M: polyclonal/monoclonal

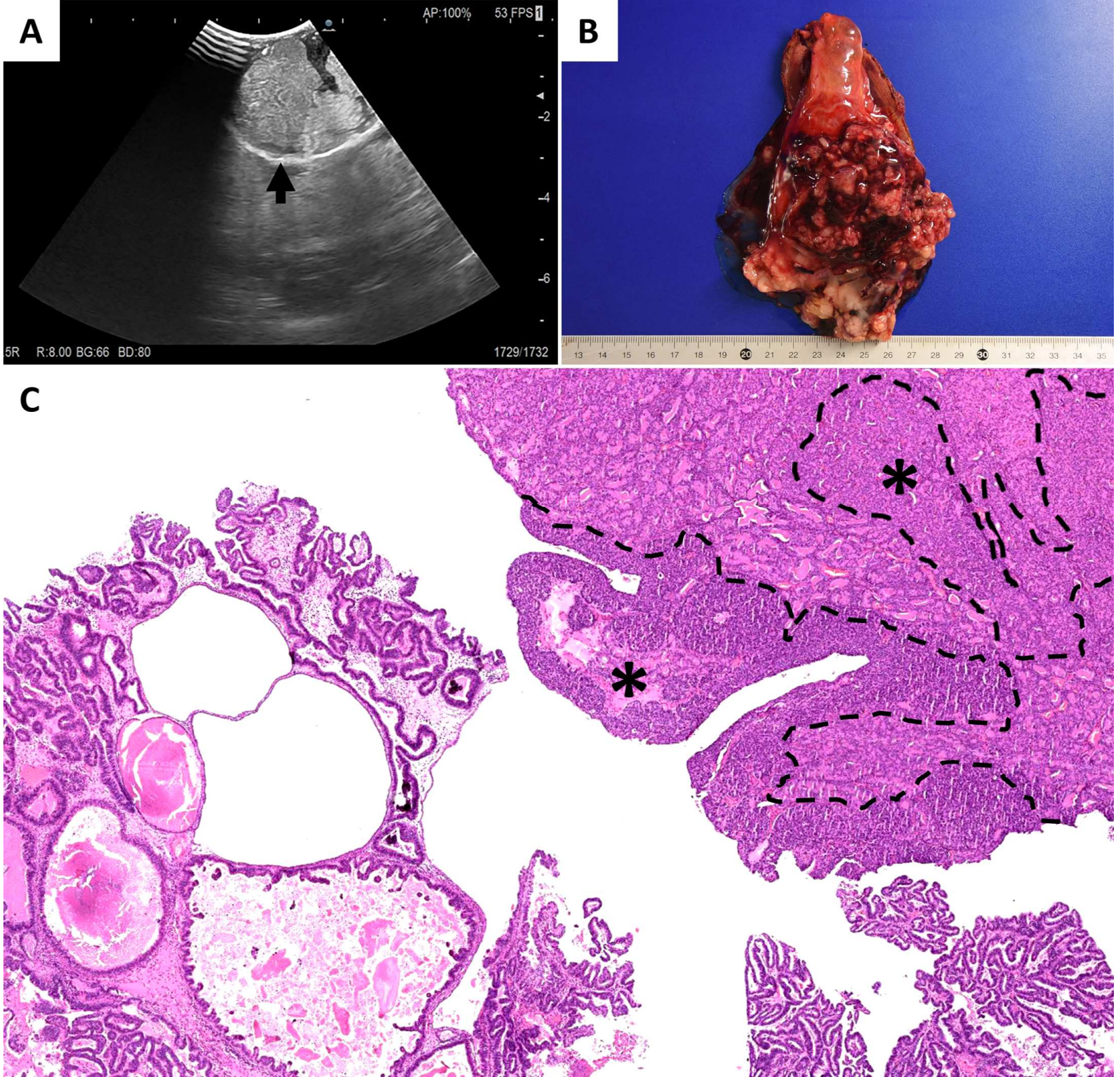
**Table 2. Review of the literature: clinico-pathological characteristics of patients with gallbladder MiNENs.**

| First author | Year | Sex | Age | Clinical presentation | Gallstones | Local extension | Nodal status | Metastases | Treatment | Outcome (month) | Neuroendocrine component | Non-neuroendocrine component |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cavazzana | 1991 | F | 71 | Jaundice | - | Limited to GB | + | - | Surgery | DOD, 4 | SCNEC | Well differentiated ADK |
| Duan | 1991 | M | 70 | Anorexia, nausea | - | Limited to GB | + | + | Surgery | DOD, 1 | SCNEC | ADK |
| Iida | 1992 | F | 62 | Abdominal pain, jaundice | - | Liver, duodenum infiltration | NA | + | Surgery, chemotherapy | DOD, 5 | SCNEC | Adenosquamous carcinoma |
| Nishihara | 1994 | F | 71 | Abdominal pain | + | Limited to GB | + | - | Surgery | DOD, 20 | SCNEC | ADK |
| Resnick | 1994 | F | 49 | Abdominal pain | - | Limited to GB | NA | NA | Surgery | NA | NET | ADK |
| Moskal | 1999 | F | 69 | Abdominal pain | - | Serosal involvement | + | + | Surgery, chemotherapy | DOD, 44 | SCNEC | Poorly differentiated ADK |
| Moskal | 1999 | F | 71 | Incidental | + | Limited to GB | + | + | Surgery, chemotherapy, radiotherapy | DOD, 13 | SCNEC | Poorly differentiated ADK |
| Moskal | 1999 | M | 40 | Abdominal pain | + | Limited to GB | + | - | Surgery, chemotherapy | DF, 189 | SCNEC | Moderately differentiated ADK |
| Eriguchi | 2000 | F | 81 | Abdominal pain | + | Limited to GB | - | - | Surgery | DF, 8 | NEC | Papillary, tubular, solid, signet ring ADK |
| Papotti | 2000 | F | 50 | Symptomatic cholelithiasis | + | Limited to GB | - | - | Surgery | DF, 12 | LCNEC | Intestinal-type ADK |
| Sakaki | 2000 | F | 79 | Appetite loss | + | Limited to GB | - | - | Surgery | DF, 8 | NET | Well differentiated ADK |
| Yannakou | 2001 | F | 72 | Abdominal pain, nausea, vomiting | + | Liver inifltration | + | - | Surgery | Dead, 2 | NEC | Well differentiated ADK |
| Okamoto | 2003 | M | 70 | Abdominal pain, lymphadenopathy | - | Liver infiltration | + | - | Surgery, chemotherapy | DOD, 6 | NEC | Papillary ADK |
| Koea | 2004 | F | 68 | Abdominal pain | - | Serosal involvement | NA | + | Surgery, chemotherapy | DOD, 6 | NEC | Mucinous ADK |
| Noske | 2006 | F | 81 | Abdominal pain, weight loss, jaundice | + | Liver infiltration | NA | + | Surgery | NA | LCNEC | Moderately differentiated adenosquamous carcinoma |
| Shimizu | 2006 | M | 58 | Abdominal pain | - | Liver infiltration | - | - | Surgery | DOD, 4 | SCNEC | Well differentiated tubular ADK |
| Sośnik | 2006 | F | 56 | Jaundice | - | Limited to GB | NA | NA | Surgery | DOD, 0.33 | SCNEC | Papillary ADK |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tsuchiya | 2006 | F | 36 | Abdominal pain, nausea | - | Limited to GB | - | - | Surgery | DF, 12 | NEC | ADK |
| Oshiro | 2008 | F | 55 | Abdominal, back pain, fever | - | Limited to GB | + | - | Surgery | DF, 20 | SCNEC, LCNEC | Papillary, tubular ADK |
| Iype | 2009 | M | 85 | Anorexia, weight loss | + | Serosal involvement | NA | - | Surgery, chemotherapy | DOD, 21 | LCNEC | ADK |
| Sato | 2010 | F | 68 | Incidental | + | Liver infiltration | NA | - | Surgery | DF, 12 | LCNEC | Well differentiated tubular ADK |
| Paniz Mondolfi | 2011 | F | 48 | Abdominal pain | - | Liver infiltration | + | + | Surgery | NA | LCNEC | Papillary ADK |
| Kim | 2011 | M | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Kim | 2011 | M | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Harada | 2012 | F | 70 | NA | + | Liver infiltration | + | NA | NA | NA | SCNEC | Well differentiated ADK |
| Harada | 2012 | F | 70 | NA | - | Serosal involvement | - | NA | NA | NA | LCNEC | Papillary well differentiated ADK |
| Harada | 2012 | F | 70 | NA | + | Duodenum infiltration | - | NA | NA | NA | NET | Well differentiated ADK |
| Harada | 2012 | F | 60 | NA | + | Limited to GB | + | NA | NA | NA | SCNEC | Papillary well differentiated ADK |
| Harada | 2012 | F | 50 | NA | - | Liver infiltration | + | NA | NA | NA | LCNEC | Well differentiated ADK |
| Song | 2012 | F | 55 | Massive ascites, chronic cholecystitis | - | liver, pancreas infiltration, serosal involvement | + | - | Surgery, chemotherapy, somatostatin analogues | DF, 7 | SCNEC | ADK |
| Abe | 2013 | F | 81 | Incidental | + | Limited to GB | + | - | Surgery | DF, 48 | NEC | Papillary and tubular ADK with squamous and sarcomatoid differentiations |
| Al-Brahim | 2013 | M | 45 | Anorexia, nausea, jaundice | + | Liver infiltration | + | + | Surgery | NA | LCNEC | ADK |
| Shintaku | 2013 | M | 80 | Incidental | - | Limited to GB | - | - | Surgery | DF, 8 | NET | Tubular ADK, squamous cell carcinoma, osteosarcoma |
| Chatterjee | 2014 | F | 73 | Cholelithiasis | + | Limited to GB | - | - | Surgery, chemotherapy, radiotherapy | RD, 45 | NEC | Moderately differentiated biliary type ADK on ICPN |
| Chen | 2014 | M | 34 | Abdominal pain | - | Serosal involvement | + | - | Surgery, chemotherapy | RD, 4 | NEC | ADK |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meguro | 2014 | F | 54 | Abdominal pain | - | Limited to GB | - | - | Surgery | DF, 24 | LCNEC | Poorly differentiated biliary type ADK on ICPN |
| Acosta | 2015 | F | 55 | Abdominal pain | + | Limited to GB | - | - | Surgery | NA | LCNEC | Well differentiated intestinal-type ADK |
| Azad | 2015 | F | 62 | Incidental | - | Serosal involvement | - | - | Surgery | DF, 24 | NET | ADK with glandular and signet ring morphology |
| Kamboj | 2015 | F | 65 | NA | NA | Limited to GB | NA | + | Chemotherapy | DF, 2 | NEC | ADK |
| Liu | 2015 | F | 63 | Abdominal pain | - | Limited to GB | - | - | Surgery | DF, 12 | LCNEC | Moderately differentiated ADK |
| Jung | 2018 | F | 54 | Abdominal pain | + | Liver infiltration | - | + | Surgery | PD, 3 | LCNEC | Adenosquamous carcinoma |
| Lin | 2018 | F | 43 | Abdominal pain | - | Liver infiltration | - | - | Surgery, chemotherapy, somatostatin analogues | Dead, 21 | SCNEC | ADK |
| Ines | 2019 | F | 74 | Abdominal pain, fever | - | Limited to GB | NA | - | Surgery | DF, 7 | LCNEC | ADK |
| Skalický | 2019 | F | 56 | Abdominal pain, weight loss | - | Liver infiltration | + | - | Surgery, chemotherapy, somatostatin analogues, biological therapy | PD, 13 | SCNC | Tubular ADK |
| Present case | 2019 | F | 66 | Abdominal pain | - | Limited to GB | - | - | Surgery | DF, 5 | LCNEC | Moderately differentiated ADK on ICPN |

ADK: adenocarcinoma; DF: disease free; DOD: dead of disease; GB: gallbladder; ICPN: intracholecystic papillary-tubular neoplasms; LCNEC: large cell neuroendocrine tumor; NA: non assessed; NEC: neuroendocrine carcinoma; NET: neuroendocrine tumor; PD: progressive disease; RD: recurrent disease; SCNC: small cell neuroendocrine carcinoma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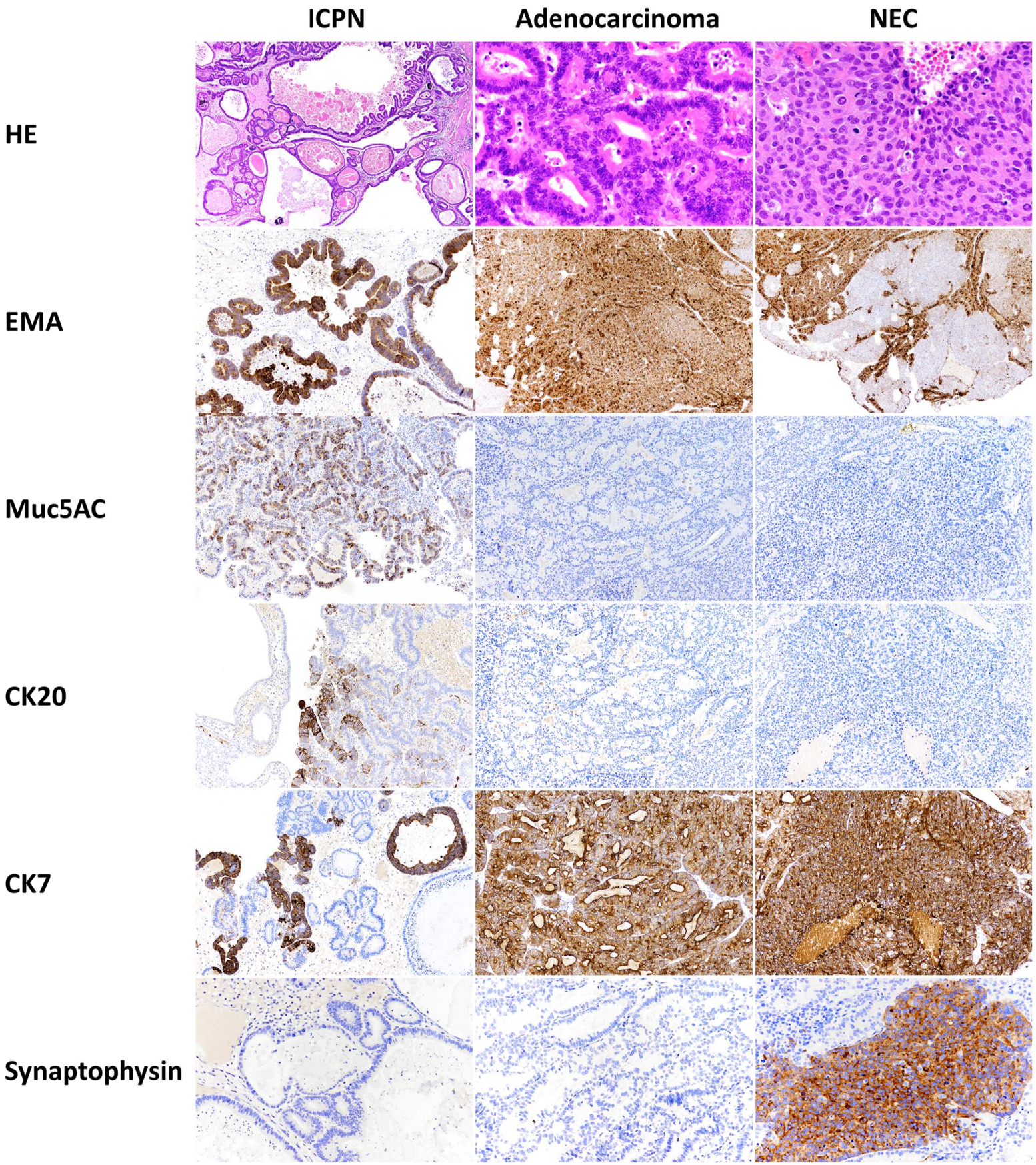
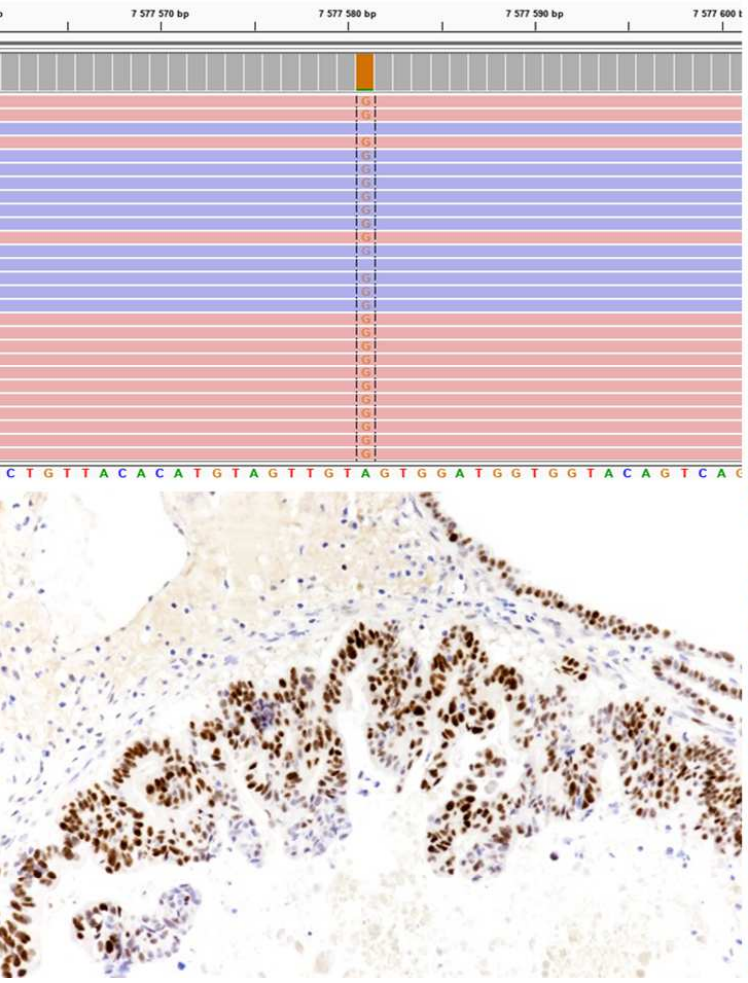
1729/1732
B
C


ICPN
Adenocarcinoma
NEC
HE
EMA
Muc5AC
CK20
CK7
Synaptophysin

## ICPN

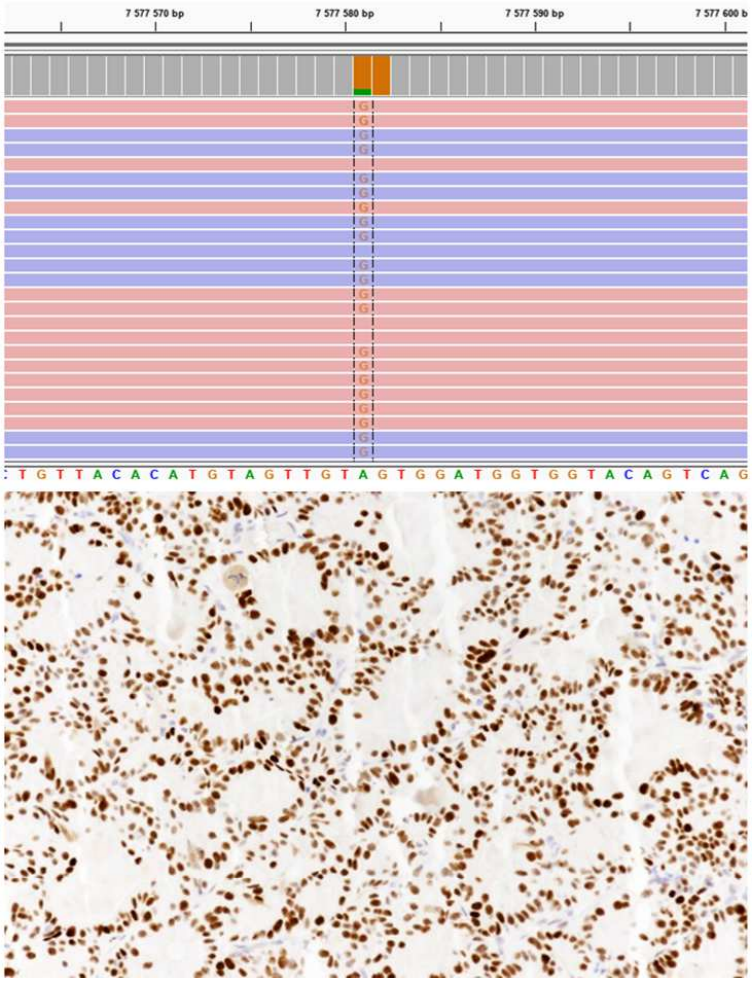
| Gene | Chr | Position | Ref. | Alt. | Read depth | Variant read depth | AF |
|---|---|---|---|---|---|---|---|
| *TP53* | 17 | 17954215 | G | T | 9279X | 12026X | 93% |






## Adenocarcinoma

| Gene | Chr | Position | Ref. | Alt. | Read depth | Variant read depth | AF |
|---|---|---|---|---|---|---|---|
| *TP53* | 17 | 17954215 | G | T | 9748X | 12799X | 83% |






## NEC

| Gene | Chr | Position | Ref. | Alt. | Read depth | Variant read depth | AF |
|---|---|---|---|---|---|---|---|
| *TP53* | 17 | 17954215 | G | T | 8122X | 10381X | 90% |