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Original Contribution

Comprehensive next generation sequencing of middle ear neuroendocrine tumors

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ABSTRACT

Middle ear neuroendocrine tumor (MeNET) is a distinctive, uncommon neoplasm of the ear. Previously regarded as "middle ear adenoma" among other names, it was found to be consistently positive for neuroendocrine markers, with differentiation analogous to normal intestinal L cells, and has therefore been classified similarly to other neuroendocrine tumors throughout the body. Nevertheless, MeNETs have an unusual two-cell population and therefore may be unique among NETs. We sought to characterize a group of MeNETs by next-generation sequencing (NGS).

Six MeNETs from the authors' archives were retrieved, with histologic and immunohistochemical results tabulated. Targeted DNA and RNA NGS were attempted on all cases. Clinical follow-up was obtained.

The MeNETs arose in the middle ears of five men and one woman, ranging from 31 to 57 years (median, 47.5 years). Four cases were grade 1 and two cases grade 2 (one based on necrosis and one based on an elevated Ki67 index). DNA NGS was successful in five of six cases, with probable pathogenic variants including: *ATRX* mutations in two cases, chromosome 22 deletion, and *DNMT3A*, *STAG2*, *RB1*, *HRAS*, *NF1*, and *SF3B1* mutations in one case each. In general, the variants were found at low allele frequencies. RNA NGS was successful in all cases, with one case harboring a fusion of unknown significance (*R3HDM2*::*EP400*). Follow up available in all cases, with five patients without disease (mean, 74 months; median, 17 months), with one patient (one of the grade 2 tumors) experiencing widespread distant metastases and dying 96 months after diagnosis.

Despite the consistent appearance of MeNET, they are heterogeneous at the molecular level, with low mutational burdens but lacking consistent, recurrent alterations. This is similar to well-differentiated NETs of other organs, in particular the small intestine and lung. Overall, our findings support the grouped classification of MeNET within the larger NET scheme.

1. Introduction

Next-generation sequencing (NGS) has had an outsized impact on classification and management of scores of head and neck tumors, including those arising in the salivary glands and sinonasal tract [1-3]. Many types of neoplasia of the ear, on the other hand, have not been thoroughly subjected to NGS, likely due to their rarity [4].

Middle ear neuroendocrine tumor (MeNET) was formerly referred to by many names such as middle ear adenoma, neuroendocrine adenoma of the middle ear, carcinoid tumor, adenomatous tumor of the middle ear, among others [5,6]. In recognition of the consistent expression of neuroendocrine markers and intestinal L cell-like differentiation, however, its classification now mirrors that of neuroendocrine tumors throughout the rest of the body [7,8]. On the other hand, MeNET is unique not only in its middle ear location, but in its two cell populations which includes a frequent abluminal layer of basal cells not seen in other neuroendocrine tumors in the body [5]. We sought to characterize a group of MeNET by NGS.

2. Materials/subjects and methods

2.1. Case selection

Following Institutional Research Ethics Board approval, MeNETs were retrieved from the authors' surgical pathology files. The cases were re-reviewed by the primary author to ensure they conformed with

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updated diagnostic criteria [8]. Histologic findings and diagnostic immunohistochemical results were tabulated.

2.2. Next-generation sequencing

Targeted RNA sequencing (RNA-Seq) and DNA sequencing was attempted on all 6 cases without matched control tissue, as described in detail elsewhere [9]. In brief, both RNA and DNA were isolated from 10 um whole-slide tissue sections using Qiagen AllPrep kits (Qiagen, Germantown, MD). Sequencing libraries are generated using Kapa Biosystems and Illumina chemistry. A custom panel of probes is used to produce an enriched DNA library containing all exons from over 1505 cancer-related genes, along with an RNA library from over 1500 genes for fusion detection. Sequencing was then performed on NextSeq 550 (Illumina), with a minimum of 6,000,000 mapped reads. All fusions and variants were reviewed in the Integrated Genomics Viewer (Broad Institute, Cambridge, MA). The Star-Fusion algorithm was used to call fusions.

3. Results

The demographic and clinical findings are summarized in Table 1. Six cases were identified. The tumors were encountered five men and one woman, ranging from 31 to 57 years (median, 47.6; mean, 46.2 years).

The immunohistochemical findings are summarized in Table 2. All cases were diffusely positive for AE1/AE3 and synaptophysin, in line with its epithelial and neuroendocrine differentiation (Fig. 1A–B). Chromogranin was positive in all five cases tested, with more variable intensity. The abluminal layer of basal cells was positive for p40 in five of six cases (Fig. 1C). SATB2 was positive in all three cases in which it was performed. Ki67 indices were very low at 1–2% in five cases (Fig. 1D), while it was clearly higher at 5% in one case.

Morphologically, the tumors were quite consistent, growing as nests and trabeculae of epithelioid cells with frequent lumen formation, in a fibrovascular stroma (Fig. 2A). The tumor cells had a plasmacytoid appearance with distinct cell borders, pale amphiphilic cytoplasm, and monotonous round nuclei exhibiting even to modestly stippled "salt-and-pepper" pattern of chromatin and indistinct nucleoli (Fig. 2B). Mitotic figures were not identified. Tumor necrosis was seen in one case.

The four least recent cases were originally diagnosed as middle ear adenoma ($n = 2$) and neuroendocrine adenoma of the middle ear (n = 2), all updated to MeNET under current criteria. The two most recent cases' diagnoses did not require revision. Four cases met criteria for grade 1 MeNET, while the tumor with necrosis and the tumor with Ki67 index of 5% were each grade 2 (Fig. 3A–B).

The molecular results are detailed in Table 3. DNA NGS was successful in five of six cases, with probable pathogenic alterations including: *ATRX* mutations in two cases, chromosome 22 deletion and *DNMT3A*, *STAG2*, *RB1*, *HRAS*, *NF1*, and *SF3B1* mutations in one case each. The probable oncogenic alterations were generally of low allele frequency. Variants of unknown significance are also listed in detail in Table 3 with their respective variant allele frequencies. RNA NGS was successful in all six cases, with only one case harboring a fusion of unknown significance (*R3HDM2*::*EP400*).

Follow up was available in all cases, with one patient initially with two recurrences, while one patient (case 3, one of the grade 2 tumors) experiencing widespread distant metastases and expiring 96 months after diagnosis.

Table 1
Clinical characteristics of middle ear neuroendocrine tumors.

Case	Age (in years)	Sex	Original diagnosis	Updated diagnosis	Follow up
1	31	M	Neuroendocrine adenoma of the middle ear	MeNET, grade 1	Re-excision at 5 months; recurrence at 10 months, managed with ossicular resection; 165 months, no evidence of disease
2	44	F	Neuroendocrine adenoma of the middle ear	MeNET, grade 1	170 months, no evidence of disease
3	57	M	Middle ear adenoma	MeNET, grade 2 (necrosis)	Subtotal resection with persistence, distant metastases to bone at 72 months, died of disease at 96 months
4	52	M	Middle ear adenoma	MeNET, grade 1	10 months, no evidence of disease
5	51	M	MeNET, grade 1	No change	17 months, no evidence of disease
6	40	M	MeNET, grade 2 (Ki67 index)	No change	Subtotal resection with persistence, re-resection at 8 months; 9 months, no evidence of disease

M = male; F = female; MeNET = middle ear neuroendocrine tumor.

4. Discussion

Middle ear neuroendocrine tumor (MeNET) is a rare neoplasm arising from the middle ear mucosa. Its varying terminology over the years (e.g., middle ear adenoma, middle ear carcinoid tumor, adenomatous tumor of the middle ear) reflects its uniqueness: it shows both glandular and neuroendocrine differentiation. Recently its classification was subsumed into the larger NET classification due to some significant similarities with NETs in other parts of the body, including consistent expression of neuroendocrine markers, a carcinoid-like histologic appearance, and L cell-like differentiation [8]. On the other hand, the biphasic nature of MeNET with a frequent abluminal layer of basal cells, is unique among NETs [8]. We sought to investigate the molecular underpinnings of MeNET, in particular to see how they compare with other, better-characterized NETs in other sites.

Our cohort was admittedly limited, as MeNET is a rare tumor. The NGS results found that MeNETs do not have a recurrent, defining genetic signature. They had an overall low mutational burden, with relatively few probable oncologic variants which were largely at low variant allele frequencies. Copy number alterations and fusions were also very limited. Accordingly, MeNETs appear to be molecularly low-complexity tumors. There was no consistent specific oncogenic signaling pathway involved; rather, the identified molecular alterations were heterogeneous, frequently involving chromatin modifiers, transcriptional regulators, and epigenetic machinery, including variants in *ATRX*, *STAG2*, *DNMT3A*, *TET1*, *SUZ12*, *KMT2D*, and *KMT2B*. The alterations were non-recurrent and often subclonal with low variant allele frequencies, therefore possibly representing secondary events rather than initiating drivers of tumorigenesis. Despite sharing in common a biphasic arrangement of two cell types with ceruminous gland adenoma, none of the MeNETs harbored *HMGA2* fusions, underscoring their distinctness from that external ear neoplasm [4].

When comparing the molecular findings of MeNET with other, better-characterized NETs in the body, some similarities can be found. The overall relative genetic simplicity of our MeNET was reminiscent of small intestinal NETs and lung grade 1 NETs (typical carcinoids), which are known for low mutational burdens [10-12]. Small intestinal NETs lack recurrent genetic drivers, while lung grade 1 NETs have occasional *MEN1* alterations [11,13]. In this series, a variant of *MEN1* was seen in one case (Case 4). Pancreatic neuroendocrine tumors (PanNETs) are characterized by recurrent, truncal alterations in *MEN1*, *DAXX*, *ATRX*, and genes involved in the mTOR pathway, often accompanied by

Table 2
Immunohistochemical characteristics of middle ear neuroendocrine tumors.

Case	Pan-CK	Synaptophysin	Chromogranin	p40	SATB2	Ki67
1	+	+	+	+ abluminal	ND	1–2%
2	+	+	ND	+ abluminal	+	1–2%
3	+	+	F+	+ abluminal	+	1–2%
4	+	+	F+	+ abluminal	ND	1–2%
5	+	+	F+	+ abluminal	ND	1–2%
6	+	+	+	–	+	5%

CK, cytokeratin; ND, not done; F, focal.

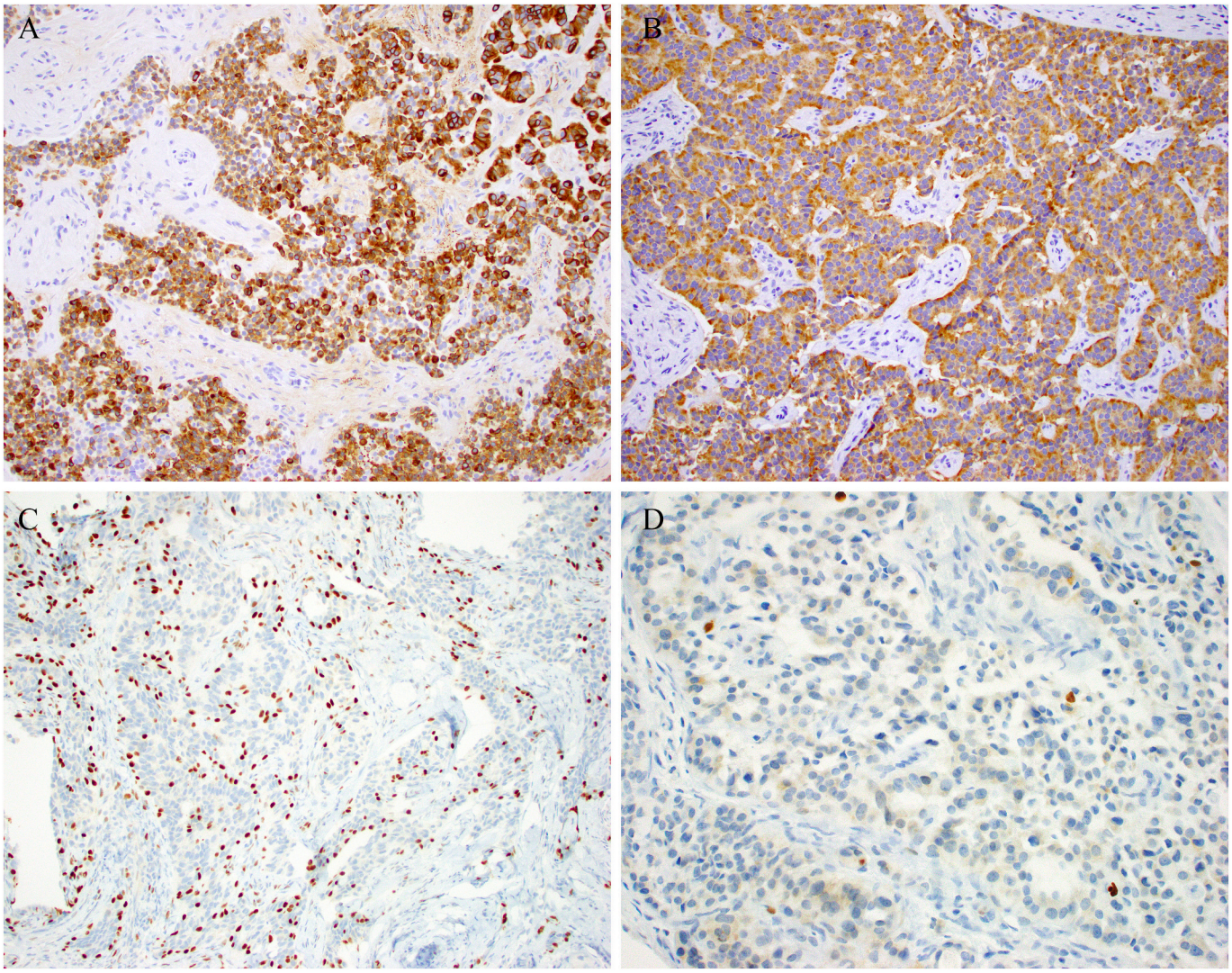

Fig. 1. The MeNETs were consistently positive for AE1/AE3 (A) and synaptophysin (B). Most cases had an abluminal pattern of expression for p40 (C) and a very low Ki67 index of 1–2% (D).

extensive copy number changes [14,15]. In addition to the *MEN1* variant in Case 4, two of our cases harbored *ATRX* mutations (Case 1 and Case 4); on the other hand, our MeNETs lacked consistent mTOR-pathway driver mutations, and a single copy number alteration was found in only one case. Despite most MeNET having an intestinal L cell-like immunophenotype, the MeNETs in this series did not closely genetically resemble well-differentiated rectal NETs, most of which recapitulate, are thought to arise from, L cells. Rectal NETs harbor frequent pathway-level alterations, including involvement of Wnt/β-catenin and PI3K–AKT–mTOR signaling [16,17].

One tumor in this series (Case 3) developed metastatic disease. While rare, this is not unheard of, as metastatic disease has been documented for MeNET [18]. It is notable that Case 3 harbored a truncating *RB1* alteration at low variant allele frequency. Alterations of *RB1* are very uncommon in well-differentiated NETs but have been described in higher-grade neuroendocrine neoplasms, including lung grade 2 NETs (atypical carcinoids) and neuroendocrine carcinomas, where they are believed to be acquired as part of transformation to a higher-grade tumor [11,19,20]. Indeed, the low variant allele frequency of the *RB1* alteration in Case 3 suggests a subclonal event, acquired during tumor progression. It will have to be seen if this finding correlates with aggressive behavior in future cases.

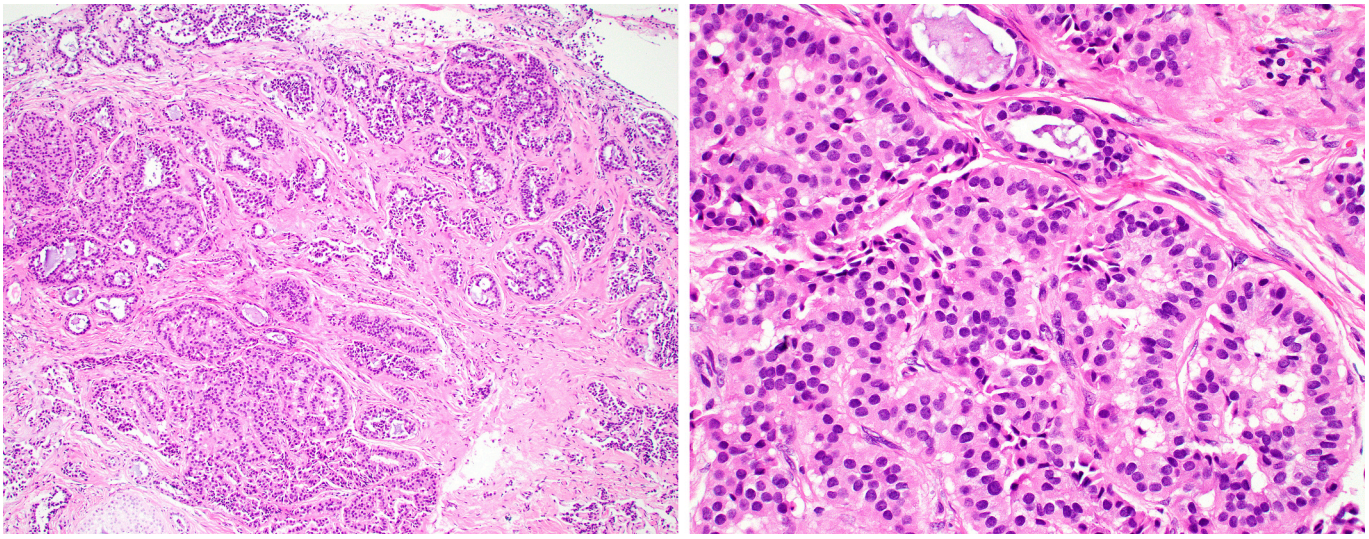

Fig. 2. The MeNETs grew as trabeculae and nests with frequent lumen formation, in a fibrous stroma (A). The tumor cells were plasmacytoid in appearance, with eccentric pale eosinophilic cytoplasm and uniform round nuclei lacking appreciable mitotic activity (B).

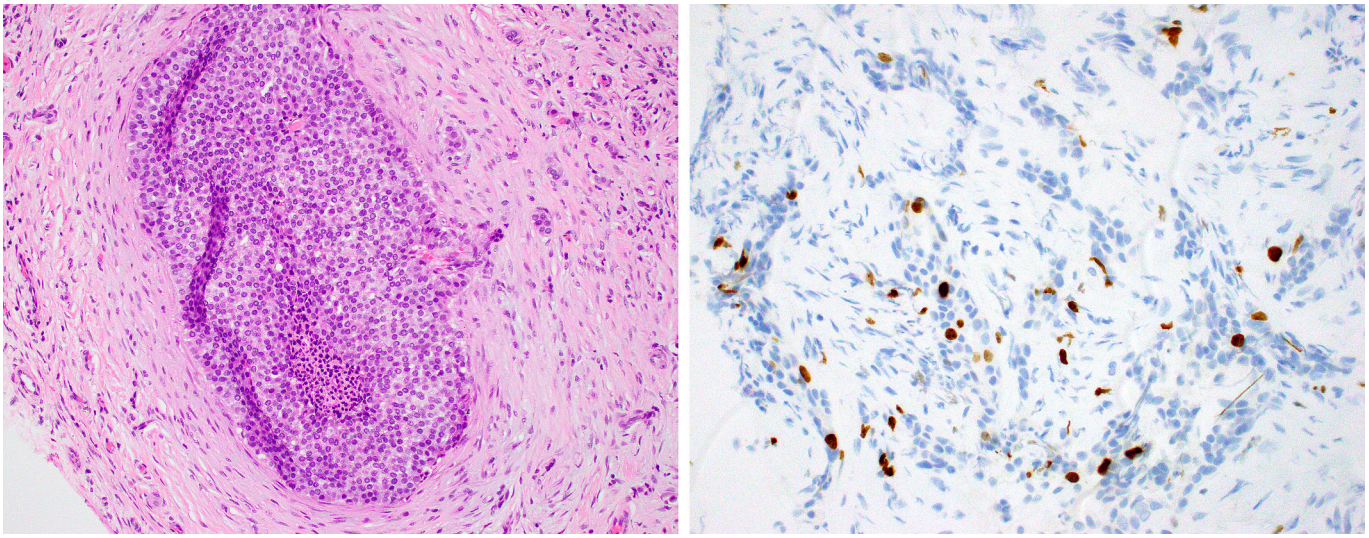

Fig. 3. One MeNET had focal necrosis (A) and another elevated Ki67 index of about 5% (B). Both were accordingly graded as grade 2 MeNETs.

In summary, this study provides the first comprehensive NGS characterization of MeNETs, albeit in a small cohort. Our findings demonstrated that MeNETs are molecularly low-complexity neoplasms lacking recurrent oncogenic drivers. Their molecular profile has some overlapping features with other NETs of the body. When coupled with histologic and immunohistochemical similarities, the NGS findings support classification in the broader NET scheme. Rare subclonal alterations affecting *RB1* may contribute to aggressive behavior in exceptional cases.

Consent to participate/publication

The IRB-approved study did not require informed consent.

CRediT authorship contribution statement

Justin A. Bishop: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft. **Jing Xu:** Conceptualization, Data curation, Writing – review & editing. **Lester D.R. Thompson:** Conceptualization, Data curation, Formal analysis, Writing – review & editing.

Ethics approval

All procedures performed in this retrospective data analysis involving human participants were in accordance with the ethical standards of the institutional review board (UT Southwestern IRB 112017-073).

Code availability

Not applicable.

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Table 3
Next-generation sequencing results for middle ear neuroendocrine tumors.

Case	Variants of possible clinical significance (VAF)	Copy number alterations	Fusions	Variants of uncertain clinical significance (VAF)
1	*ATRX* p.Arg907* (3.08%)	None	None	*CENPF* p.Glu2694fs (41.80%) *BACH1* p.Gln331Arg (45.45%) *PDGFRB* p.Lys304Arg (36.93%) *GPHN* p.Leu42Val (41.85%) *MLLT10* p.His565Gln (40.67%) *BRWD3* p.Arg935His (7.19%) *PPFIA2* p.Arg1204His (37.61%) *CDK9* c.266-7G>A (42.06%) *PCM1* c.783+303C>T (38.91%) *CBFA2T3* p.Ser189Thr (50.24%)
2	None	chr22 p11.2-q13.33 deletion	None	*IKBKE* p.Arg229Trp (35.20%) *FGFR3* p.Pro401Ser (59.62%) *TRIP11* p.Gly7del (46.19%) *GBP2* p.Val177Ala (50.58%) *LAMA1* p.Val1575Ile (37.25%) *ERBB2* p.Leu1061Pro (50.99%) *ICAM1* p.Asp98Asn (46.51%) *MAP3K13* p.Gln99Pro (51.60%) *HIST1H1C* p.Val88Met (46.08%) *FOSL1* p.Arg169Gln (41.71%) *PTPRO* p.Ile698Val (44.00%) *NGFR* p.Ala225Gly (50.00%) *ERG* p.Ile311Phe (23.35%) *RECQL4* p.Arg1064Cys (42.13%) *CBLB* p.Met1? (46.41%) *MAP3K13* p.Arg837His (44.16%)
3	*DNMT3A* p.Arg882His (3.22%) *STAG2* p.Arg953* (4.60%) *RB1* p.Trp563* (3.29%)	None	None	*CTNND2* p.Gly1101Arg (48.45%) *CBLB* p.Leu190Pro (40.83%) *RECQL* p.Ile497fs (51.59%) *ITGA5* c.219-17_219-5delG CTTTTCCCCCCCinsACTTTTCCCCCCA (13.73%) *SLCO1B3* p.Val560Val (7.97%) *ELL* p.AlaGln348ValLys (36.53%) *GANAB* p.Thr271Thr (35.57%) *CBLB* c.445+173C>T (43.58%) *ERBB4* p.Pro574Leu (4.85%) *EXT2* p.Ala434Val (31.07%) *KAT6A* p.Arg366* (3.25%) *EGR2* p.Ala309dup (29.81%) *PCM1* p.Leu371Phe (40.70%) *CTNNB1* p.Pro714Pro (41.62%) *SUGP2* p.Glu895del c6 (50.18%) *GRID1* p.Met430Val (30.81%) *PIK3C2G* p.Val897Ile (23.31%) *CAMTA1* p.Thr33Ser (40.76%) *ERCC3* p.Pro400Ser (38.13%) *MKI67* p.Asp1321Gly (42.77%)
4	*ATRX* p.Lys1471_Glu1519del (11.27%) *HRAS* p.Thr58Ile (4.76%) *NF1* c.7970+1G>A (6.50%)	None	None	*SLC45A3* p.Leu223_Ala234del (24.54%) *MEN1* p.ProThr540SerAla (50.18%) *AKAP9* p.Met2525Ile (33.87%) *FANCB* p.Pro392Ser (11.65%) *PTPRS* p.Thr1501Ala (37.32%) *LMO7* p.Arg582Gln (56.79%) *APC* p.Arg564Gln 44.06% *PRDM7* p.LeuArg130SerLys 18.18% *LAMA5* p.Pro3168Leu 36.14% *CENPF* p.Arg2309Ser 33.70% *TET1* p.Pro1969Ser 5.98% *RICTOR* p.Arg380Cys 11.02% *FLT1* p.Glu645Lys 24.59% *BARD1* p.Leu359_Pro365del (27.50%) *STK11* c.1109-4C>T (45.35%) *HSP90AA1* p.Met1? (42.76%) *DAB2IP* p.Gln579Arg (34.97%) *JAG2* p.Thr841Ala (43.42%) *CIT* p.Glu1055Asp (43.68%) *CEP170B* p.Leu110Leu (33.62%) *HOXC11* p.His125Arg (39.73%)
5	DNA sequencing failed	DNA sequencing failed	*R3HDM2*::*EP400*	DNA sequencing failed
6	SF3B1 p.Lys666Asn (20.43%)	None	None	*ZFHX3* p.Thr2724Ser (45.27%) *SUZ12* p.Ala231Gly (49.23%) *PTCRA* p.Ala176Thr (42.01%)

(*continued on next page*)

Table 3 (*continued*)

Case	Variants of possible clinical significance (VAF)	Copy number alterations	Fusions	Variants of uncertain clinical significance (VAF)
				BAZ2A p.Pro675Arg (41.35%) *SLIT2* p.Pro1494Leu (38.22%) *PIK3CD* p.Val445Ile (41.35%) *MAP2K4* p.Val375Ile (65.96%) *NCKIPSD* p.Pro551Ser (42.49%) *PDE4DIP* p.Arg401Gln (28.86%) *PTPN2* p.Glu321Lys (33.33%) *COL11A1* c.2295+6G>A (38.53%) *USP42* p.Thr205Ala (35.57%) *WDR90* p.Ile945Met (54.62%) *RASGRF1* p.Tyr787His (49.22%) *TNFRSF17* p.Ile55Asn (33.06%) *NCOR1* p.His1563Arg (49.67%) *NUMA1* p.Arg1270His (47.09%) *PCLO* p.Pro2557Ser (42.86%) *KMT2D* p.Arg4960Gln (40.53%) *BCL11B* p.Gly83Ser (47.76%) *KMT2B* p.Pro2292Ser (43.54%) *FLCN* p.Cys82Gly (47.53%)

VAF, variant allele frequency.

Declaration of competing interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

References

[1] Bishop JA. Fusions in salivary gland neoplasms: a review of practical diagnostic applications. J Clin Pathol 2025;78(5):289–97.
[2] Skalova A, Stenman G, Simpson RHW, et al. The role of molecular testing in the differential diagnosis of salivary gland carcinomas. Am J Surg Pathol 2018;42(2):e11–27.
[3] Thompson LDR, Bishop JA. Update from the 5th edition of the World Health Organization classification of head and neck tumors: nasal cavity, paranasal sinuses and skull base. Head Neck Pathol 2022;16(1):1–18.
[4] Rooper L.M., Thompson L.D.R., Sangoi A.R., et al. Targeted molecular profiling uncovers true ceruminous adenomas with HMGA2::WIF1 and ceruminous syringocystadenoma papilliferum with BRAF V600E. Virchows Arch 2025. Online ahead of print.
[5] Lott Limbach AA, Hoschar AP, Thompson LD, et al. Middle ear adenomas stain for two cell populations and lack myoepithelial cell differentiation. Head Neck Pathol 2012;6(3):345–53.
[6] Torske KR, Thompson LDR. Adenoma versus carcinoid tumor of the middle ear: a study of 48 cases and review of the literature. Mod Pathol 2002;15(5):543–55.
[7] Asa SL, Arkun K, Tischler AS, et al. Middle ear "adenoma": a neuroendocrine tumor with predominant L cell differentiation. Endocr Pathol 2021;32(4):433–41.
[8] Bell D, Asa SL, Lott Limbach AA, et al. Middle ear neuroendocrine tumour. In: Board WcoTE, editor. WHO classification of head and neck tumours. Lyon, France: IARC Press; 2024. p. 448–50.
[9] Bishop JA, Gagan J, Baumhoer D, et al. Sclerosing polycystic "adenosis" of salivary glands: a neoplasm characterized by PI3K pathway alterations more correctly named sclerosing polycystic adenoma. Head Neck Pathol 2020;14(3):630–6.
[10] Samsom KG, van Veenendaal LM, Valk GD, et al. Molecular prognostic factors in small-intestinal neuroendocrine tumours. Endocr Connect 2019;8(7):906–22.
[11] Fernandez-Cuesta L, Peifer M, Lu X, et al. Frequent mutations in chromatin-remodelling genes in pulmonary carcinoids. Nat Commun 2014;5:3518.
[12] Simbolo M, Mafficini A, Sikora KO, et al. Lung neuroendocrine tumours: deep sequencing of the four World Health Organization histotypes reveals chromatin-remodelling genes as major players and a prognostic role for TERT, RB1, MEN1 and KMT2D. J Pathol 2017;241(4):488–500.
[13] Laddha SV, da Silva EM, Robzyk K, et al. Integrative genomic characterization identifies molecular subtypes of lung carcinoids. Cancer Res 2019;79(17):4339–47.
[14] Scarpa A, Chang DK, Nones K, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature 2017;543(7643):65–71.
[15] Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 2011;331(6021):1199–203.
[16] Duan X, Zhao M, Yin X, et al. Molecular typing and mutational characterization of rectal neuroendocrine neoplasms. Cancer Med 2023;12(15):16207–20.
[17] Gastrointestinal Pathology Study Group of Korean Society of P, Sohn JH, Cho MY, et al. Prognostic significance of defining L-cell type on the biologic behavior of rectal neuroendocrine tumors in relation with pathological parameters. Cancer Res Treat 2015;47(4):813–22.
[18] Engel MSD, Hensen EF, Bloemena E, et al. Metastastic potential of middle ear neuroendocrine tumours. Acta Otorhinolaryngol Ital 2025;45(6):431–6.
[19] Rekhtman N, Pietanza MC, Hellmann MD, et al. Next-generation sequencing of pulmonary large cell neuroendocrine carcinoma reveals small cell carcinoma-like and non-small cell carcinoma-like subsets. Clin Cancer Res 2016;22(14):3618–29.
[20] Joseph NM, Umetsu SE, Kim GE, et al. Progression of low-grade neuroendocrine tumors (NET) to high-grade neoplasms harboring the NEC-like co-alteration of RB1 and TP53. Endocr Pathol 2024;35(4):325–37.