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Case Report

Non-*C19MC*-altered embryonal tumor with multilayered rosettes in a young woman with DICER1 syndrome: case report and review of the literature

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Summary

Embryonal tumors with multilayered rosettes (ETMR) are highly aggressive and therapy-resistant pediatric central nervous system (CNS) tumors that have three histological patters: embryonal tumor with abundant neuropil and true rosettes, ependymoblastoma, and medulloepithelioma. We present a case of ETMR in an 18-year-old woman with DICER1 syndrome. This report confirms the important role of DNA-methylation analysis in the classification of CNS embryonal tumors and the importance of investigating somatic and germline *DICER1* mutations in all CNS embryonal tumors.

Key words: DICER1 syndrome, embryonal tumor with multilayered rosettes, *DICER1*, DNA methylation-based CNS tumor classifier

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Case report

We report the case of an 18-year-old woman who was admitted to the emergency room for disorientation and incoherent speech. Her relevant medical history included papillary thyroid carcinoma at age 13, treated by total thyroidectomy. Magnetic resonance imaging (MRI) revealed a 37x27x34-mm sellar and suprasellar solid tumor suggestive of pituitary macroadenoma (Fig. 1). Hormone blood tests were negative. The mass was subtotally removed. Histology showed infiltration of the pituitary gland by a tumor composed of small immature cells with hyperchromatic round-to-oval nuclei and scant cytoplasm that molded and focally formed pseudo-rosettes on a background myxoid stroma (Fig. 2A, B, C). Apoptosis and necrosis were identified (Fig. 2D). Tumor cells showed

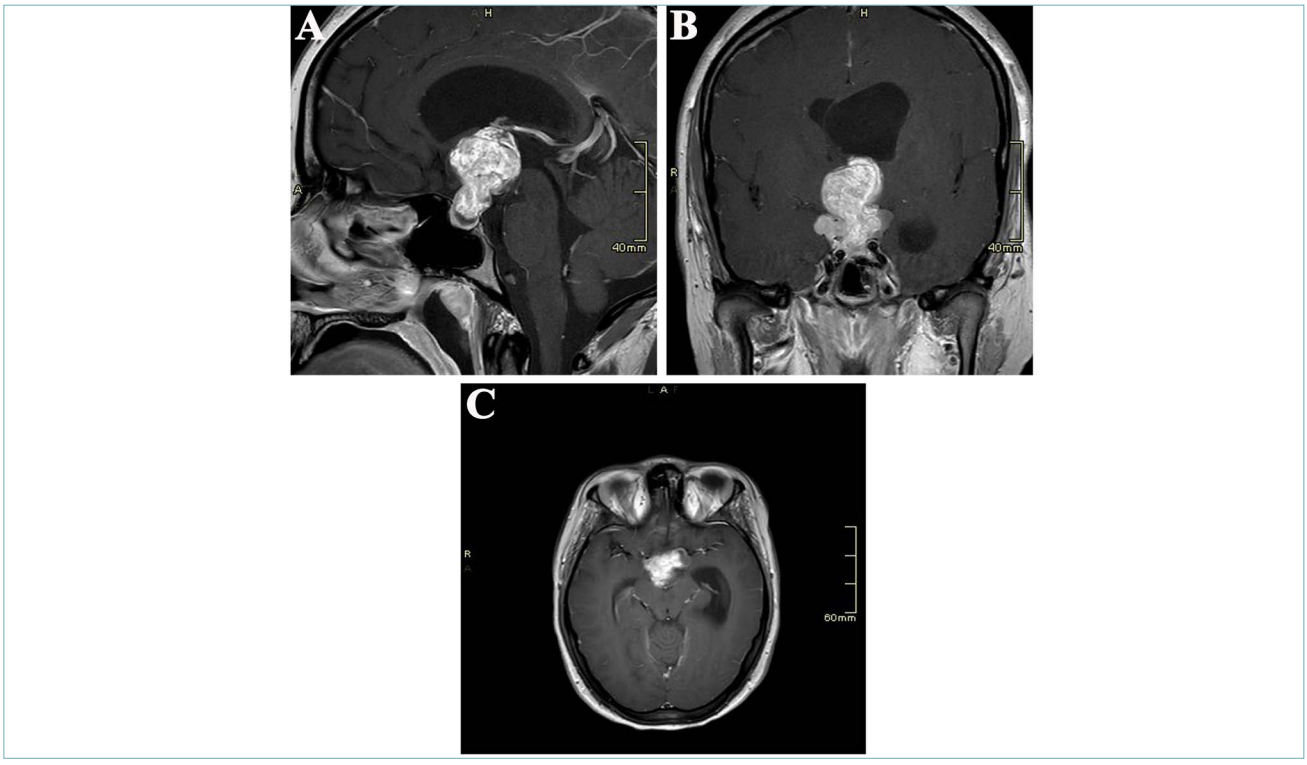

Figure 1. Sagittal (A), coronal (B), and axial (C) postcontrast T1-weighted MRI showed a large solid sellar mass which extended supratentiorally.

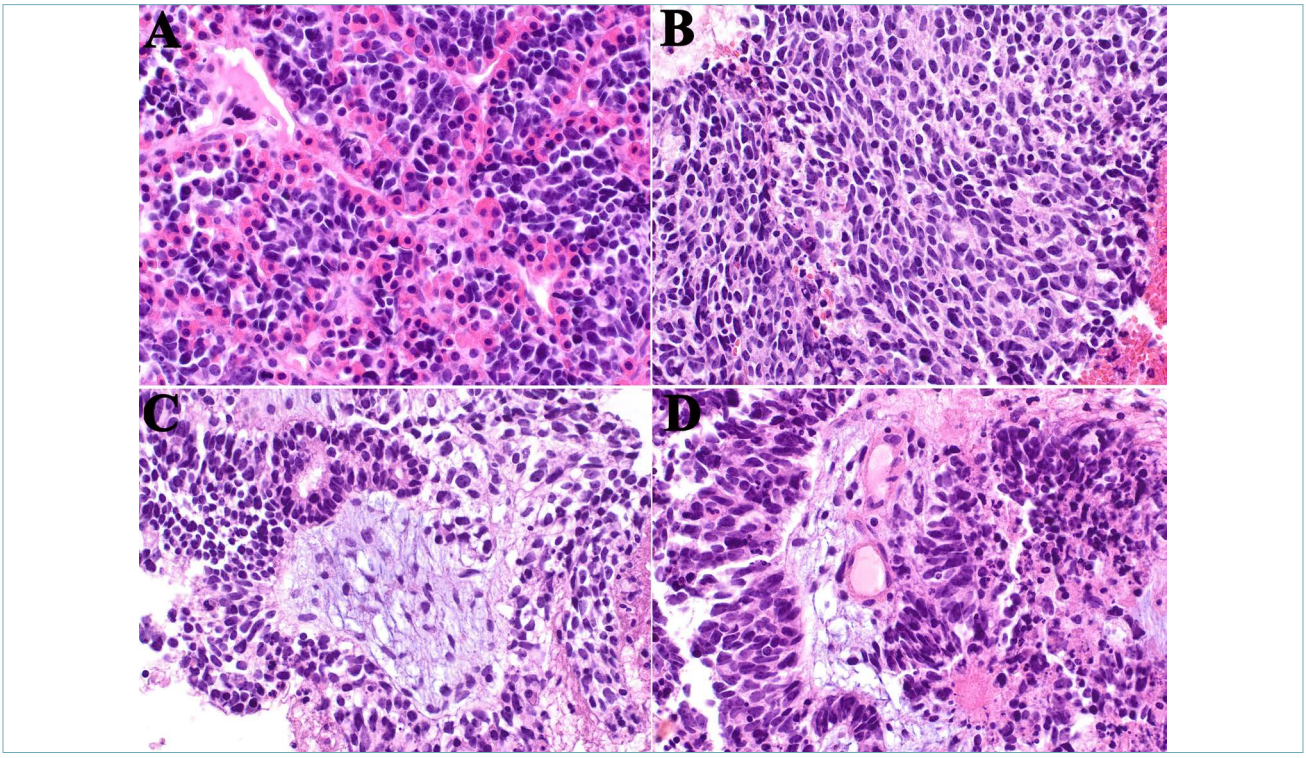

Figure 2. H&E showed small round undifferentiated tumor cells with normal pituitary secretory cells (A). The tumor has a diffuse growth pattern (B) with occasional pseudo-rosettes (C). Mitoses are abundant and necrosis and apoptotic figures are easily identified (D).

diffuse positivity with CD56 and synaptophysin, and stained only focally with NF, cytokeratin AE1/AE3 (Fig. 3A) and cytokeratin 8/18. SMARCB1 (INI1) expression was retained. Strong nuclear labeling with p53 was observed in 70% of tumor cells (Fig. 3B). CD45, GFAP, myogenin, chromogranin, EMA and pituitary hormones (ACTH, GH, LH, FSH, TSH and prolactin) were negative. The proliferative index (Ki-67) was high (85%) (Fig. 3C). Considering the tumor's morphology, immunophenotype and location, pituitary blastoma, embryonal tumor with multilayered rosettes (ETMR), and CNS embryonal tumor NOS were the three entities entered in the differential diagnosis. Based on the histopathological features of the tumor and the patient´s medical history of metachronous papillary thyroid carcinoma, gene sequencing with a 101-gene NGS panel was performed ("Hereditary Plus Oncokit"; Health in Code) (Tab. I). We identified a frameshift vari-

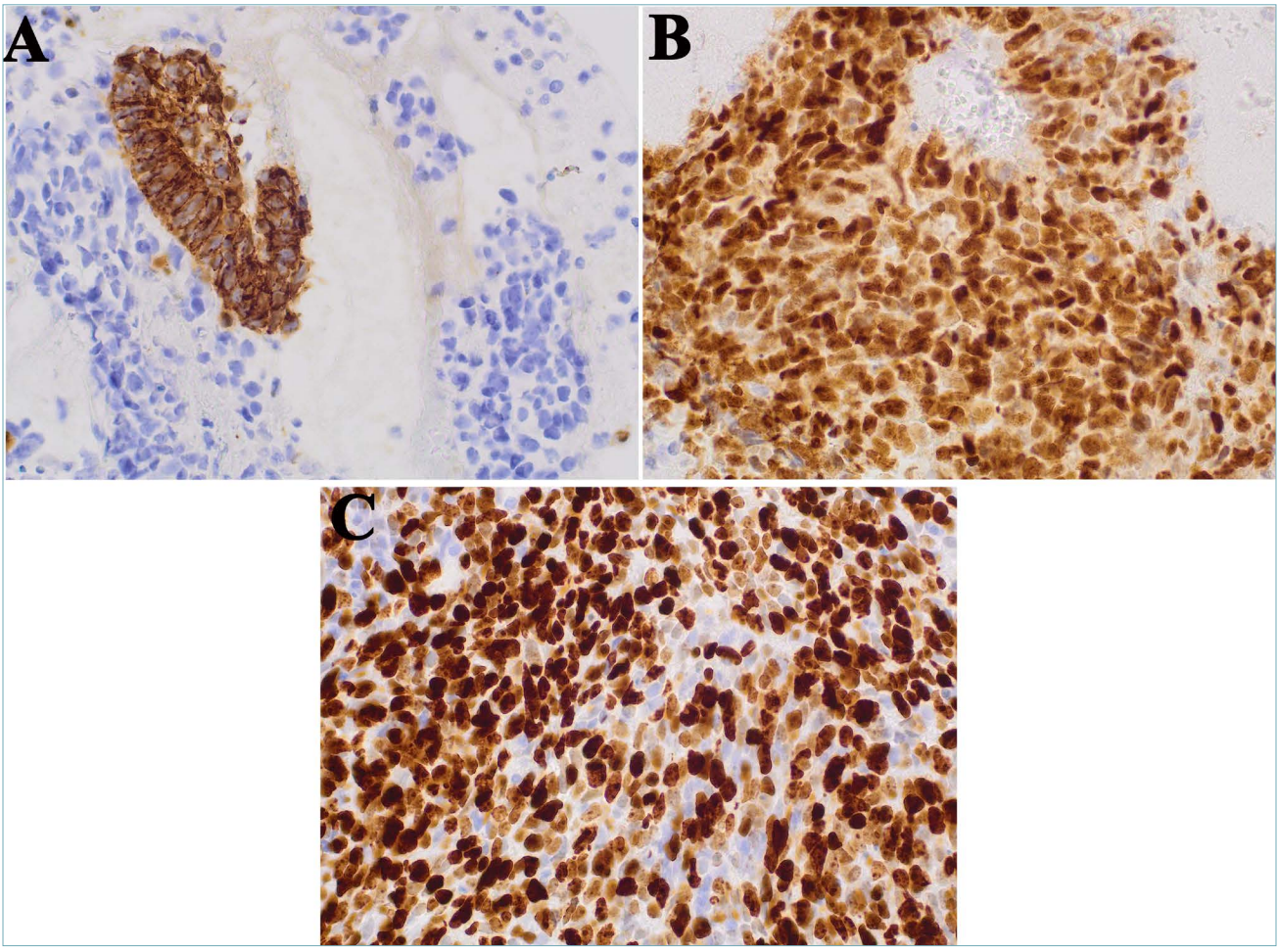

Figure 3. Focal expression of cytokeratin AE1/AE3 (particularly in the pseudo-rosettes) (A). P53 immunostaining (B) and Ki-67 immunostaining (C).

Table I. Hereditary cancer global panel genes.

Hereditary cancer global panel genes
ABRAXAS1 (FAM175A), ACD, AIP, AKT1, ALK, APC (incl. 5' UTR), ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CHEK2, CTNNA1, CYLD, DICER1, EPCAM (incl. 3' UTR), FANCC, FANCG, FANCM, FH, FLCN, GALNT12, GALNT14, GDNF, GEN1, GREM1, HABP2, HOXB13, KIF1B, KLLN, LZTR1, MAX, MC1R, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PHOX2B, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RB1, RECQL4, RET, RINT1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEC23B, SLX4, SMAD4, SMARCA4, SMARCB1, SMARCE1, SPRED1, STK11, SUFU, TERF2IP, TERT, TMEM127, TP53, TSC1, TSC2, VHL, WT1 and XRCC2.

ant (NM_030621.4:c.5487_5488insA, p.Val1830Serfs) and a hotspot (hs) missense mutation within the RNAse IIIb domain of *DICER1* (NM_030621.4:c.5127T>A, p.Asp1709Glu), which narrowed down the differential diagnosis, as pituitary blastomas and rare ETRMs (5%) are known to harbor *DICER1* mutations. In order to determine a final diagnosis, supplementary DNA methylation-based analysis (Illumina 850k EPIC array) was performed, and data was analyzed using the Heidelberg CNS tumor classifier. The classifier produced a class prediction consistent with ETMR, non-*C19MC*-altered (calibrated score of 0.97). Using unsupervised t-SNE analysis together with a previously published and publicly available dataset of DICER1 associated neoplasms, [1] the tumor clustered with ETMR and ciliary body medulloepithelioma, and away from other CNS tumors known to be associated with DICER1 syndrome, such as pineoblastoma and primary intracranial sarcoma with DICER1 alteration (Fig. 4A). Array-based copy number profiling identified panchromosomal copy-number alterations. In line with the DNA methylation class prediction, no amplification of the *C19MC* locus was identified (Fig. 4B). A final diagnosis of ETMR, non-*C19MC*-altered was rendered and the patient underwent treatment with 6 cycles of adjuvant chemotherapy (etoposide/cisplatin) and sequential radiation therapy with radical intent. Control MRI performed upon treatment completion showed stable disease. The patient was then referred to genetic counselling, where a germline mutation of the frameshift *DICER1* variant (NM_030621.4:c.5487_5488insA, p.Val1830Serfs) was shown, thus confirming DICER1 syndrome.

Retrospective sequencing of the thyroid carcinoma diagnosed 5 years earlier identified a hs *DICER1* alteration (NM_030621.4:c.5125G>A p.Asp1709Asn) alongside

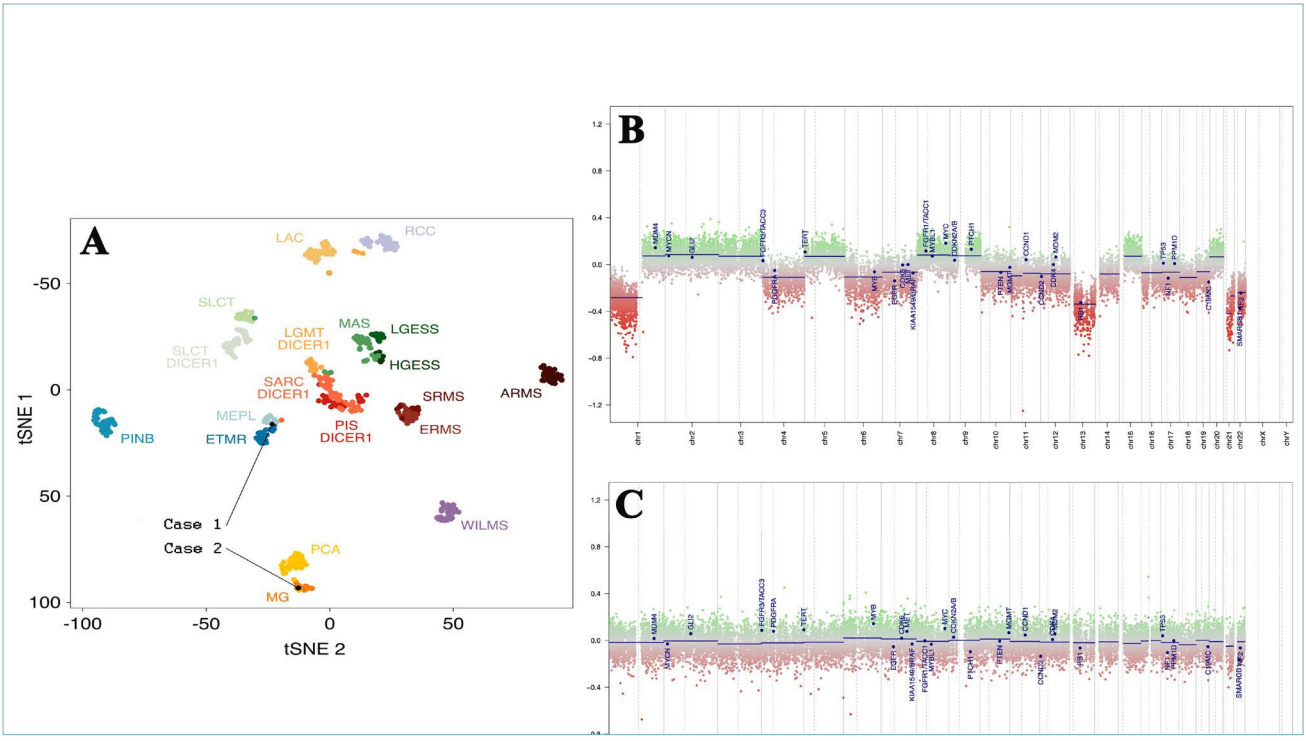

Figure 4. t-SNE plot of DNA methylation data including the patient's brain tumor (case 1) and the thyroid cancer (case 2) together with a large dataset of DICER1 associated neoplasms.[1] The brain tumor (Case 1) clustered with embryonal tumor with multilayered rosettes (ETMR) and ciliary body medulloepithelioma (MEPL). The thyroid tumor (Case 2) clustered with papillary thyroid carcinoma (PCA) and multinodular goiter (MG) (A). Multiple copy number alterations are identified in the cerebral tumor, however no *C19MC* amplification is noted (B). No copy number variations were found in the thyroid tumor (C). WILMS: Wilms tumor; SRMS: MYOD1-mutant spindle cell and sclerosing rhabdomyosarcoma; ARMS: alveolar rhabdomyosarcoma; LGESS: low-grade endometrial stromal sarcoma; HGESS: high-grade endometrial stromal sarcoma; MAS: Müllerian adenosarcoma; LAC: lung adenocarcinoma; RCC: clear cell renal cell carcinoma; LGMT DICER1: low-grade mesenchymal tumor with DICER1 alteration; SARC DICER1: sarcoma with DICER1 alteration; PIS DICER1: primary intracranial sarcoma with DICER1 alteration; PINB: pineoblastoma; SLCT: ovarian Sertoli-Leydig cell tumor; ERMS: embryonal rhabdomyosarcoma.

the germline variant (NM_030621.4:c.5487_5488insA p.Val1830Serfs), which proved this carcinoma to be part of the syndrome phenotype. As expected, DNA methylation-based analysis of the thyroid tumor showed co-clustering with papillary thyroid carcinoma and multinodular goiter (Fig. 4A). No significant copy number variations were detected in the thyroid sample (Fig. 4C).

Discussion

DICER1 syndrome is an uncommon tumor predisposition syndrome of autosomal dominant inheritance caused by a heterozygous germline *DICER1* loss-of-function pathogenic variant followed by somatic missense mutations on the wild-type allele. DICER1 syndrome is characterized by its reduced penetrance with no clear genotype-phenotype correlation [2]. Pleuropulmonary blastoma, the most common primary lung malignancy in children, is a highly distinctive feature of the syndrome. Other manifestations are thyroid adenoma and carcinoma, pediatric cystic nephroma, embryonal rhabdomyosarcoma, ovarian Sertoli-Leydig cell tumor, and various other rare presentations [1]. In the central nervous system, DICER1-associated neoplasms typically affect children between 0 and 10 years of age, and include a series of high-grade tumors of embryonal lineage with primitive blastemal characteristics that can occur at different sites within the neuroaxis [3]. Primary CNS tumors associated with DICER1 syndrome are exceedingly rare and include pituitary blastoma (a pathognomonic manifestation of a germline *DICER1* mutation), pineoblastoma, ciliary body medulloepithelioma, DICER1-mutant primary intracranial sarcoma, and ETMR [4,5]. ETMR is a high-grade (WHO grade 4) aggressive tumor occurring almost exclusively in young children. ETMRs exhibit three main histological patterns: ependymoblastoma, medulloepithelioma, and embryonal tumor with abundant neuropil and true rosettes. These patterns are considered different morphologic variants within the same tumor entity. In our case, the presence of occasional tubular structures reminiscent of embryonic neural tubes or elongated multilayered rosettes favors medulloepithelioma. Occasionally, ETMRs may also show a pineoblastoma-like histology. In our case, pineoblastoma was excluded from the differential diagnosis due to the tumors's sellar location. Furthermore, divergent differentiation (osteoid, myeloid, epithelial, mesenchymal or muscular differentiation) has been described in ETMRs. Because of the diverse histology patterns, the diagnosis currently relies heavily on the identification of molecular alterations. Approximately 90% of ETMRs are characterized by amplification of the C19MC oncogenic miRNA cluster on chromosome 19 or C19MC fusion with *TTYH1* that results in upregulation of this miRNA cluster. Rare ETMRs (< 5%) harbor *DICER1* mutations, and all of these cases have been reported in the setting of DICER1 syndrome. Interestingly, all *DICER1* mutated ETMRs lack C19MC amplification. To this date, only 9 ETMR cases with *DICER1* mutation lacking C19MC amplification have been described in the literature [5-7]. They occurred in very young children (mean age: 1.1 years, range, 0.2-2 years) with a clear predilection for girls (6:3). Five tumors arose in the cerebellum, one in the intraventricular region with infiltration of the thalamus and the vermis of the cerebellum, and one case in the supratentorial middle region. In contrast, our case occurred in an older patient (18 years) and in an atypical location with involvement of the sellar and suprasellar region. In fact, the initial clinically suspected diagnosis was a pituitary macroadenoma. To our knowledge, this is the first case of ETMR with *DICER1* mutation in the sellar region. A case of medulloblastoma in the sellar region has been previously reported; however, no molecular studies were performed [8].

Another interesting finding revealed by the DNA methylation study is that the brain tumor in our patient clustered near ciliary body medulloepithelioma. Although ciliary body medulloepitheliomas share some histopathological characteristics with CNS ETMR, they have different molecular signatures, supporting that they are different nosologic entities.[9] However, our finding suggests that ETMR, non-*C19MC*-altered may share a common histogenesis and biological behavior with a subset of ciliary body medulloepitheliomas. Furthermore, many ciliary body medulloepitheliomas (4/7) present in the methylation dataset are reported to harbor *DICER1* mutations [1].

This report confirms the important role of DNA-methylation analysis in the classification of CNS embryonal tumors [10]. Besides prognostic implications, accurate diagnosis of these tumors is necessary for appropriate treatment [11]. Although not yet endorsed by the WHO, it is expected that this new computational tool will increasingly be integrated into the clinical workflow alongside standard molecular analyses because of their potential to accurately refine the diagnosis [12,13]. We would also like to emphasize the importance of investigating somatic and germline *DICER1* mutations in all CNS embryonal tumors. This is particularly crucial when there is a clinical history suggestive of DICER1 syndrome, such as a personal or family history of other neoplasms known to be associated with the DICER1 syndrome (e.g., thyroid lesions).

Conflict of interest

The authors declare no conflict of interest.

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None.

Authors' contributions

All authors contributed to the article and read and approved the final version of the manuscript.

Ethical consideration

The information contained in this manuscript complies with the journal's ethical standards.

References

1 Kommoss FKF, Chong AS, Chong AL, et al. Genomic characterization of DICER1-associated neoplasms uncovers molecular classes. Nat Commun. 2023;14:1677. https://doi.org/10.1038/s41467-023-37092-w

2 de Kock L, Wu MK, Foulkes WD. Ten years of DICER1 mutations: Provenance, distribution, and associated phenotypes. Hum Mutat. 2019;40:1939-1953. https://doi.org/10.1002/humu.23877.

3 Nosé V, Gill A, Teijeiro JMC, et al. Overview of the 2022 WHO Classification of Familial Endocrine Tumor Syndromes. Endocr Pathol. 2022;33:197-227. https://doi.org/10.1007/s12022-022-09705-5

4 de Kock L, Sabbaghian N, Plourde F, et al. Pitiutary blastoma: a patognomonic feature of germ-line DICER1 mutations. Acta Neuropathol. 2014;128:111-122. https://doi.org/10.1007/s00401-014-1285-z

5 de Kock L, Priest JR, Foulkes WD, et al. An update on the central nervous system manifestations of DICER1 syndrome. Acta Neuropathol. 2020;139:689-701. https://doi.org/10.1007/s00401-019-01997-y

6 Lambo S, Gröbner SN, Rausch T, et al. The molecular landscape of ETMR at diagnosis and relapse. Nature. 2019;576:274-280. https://doi.org/10.1038/s41586-019-1815-x

7 Uro-Coste E, Masliah-Planchon J, Siegfried A, et al. ETMR-like infantile cerebellar embryonal tumors in the extended morphologic spectrum of DICER1-related tumors. Acta Neuropathol. 2019;137:175-177. https://doi.org/10.1007/s00401-018-1935-7

8 Pang LM, Roebuck DJ, Ng HK, Chan YL. Sellar and suprasellar medulloepithelioma. Pediatr Radiol. 2001;31:594-596. https://doi.org/10.1007/s002470100495

9 Korshunov A, Jakobiec FA, Eberhart CG, et al. Comparative integrated molecular analysis of intraocular medulloepitheliomas and central nervous system embryonal tumors with multilayered rosettes confirms that they are distinct nosologic entities. Neuropathology. 2015;35:538-44. https://doi.org/10.1111/neup.12227

10 Perez E, Capper D. Invited Review: DNA methylation-based classification of paediatric brain tumours. Neuropathol Appl Neurobiol. 2020;46:28-47. https://doi.org/10.1111/nan.12598

11 Sturm D, Orr BA, Toprak UH, et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell. 2016;164:1060-1072. https://doi.org/10.1016/j.cell.2016.01.015

12 Kumar R, Liu APY, Orr BA, et al. Advances in the classification of pediatric brain tumors through DNA methylation profiling: From research tool to frontline diagnostic. Cancer. 2018;124:4168-4180. https://doi.org/10.1002/cncr.31583

13 Schepke E, Löfgren M, Pietsch T, et al. DNA methylation profiling improves routine diagnosis of paediatric central nervous system tumours: A prospective population-based study. Neuropathol Appl Neurobiol. 2022;48:e12838. https://doi.org/10.1111/nan.12838