Discover Oncology

https://doi.org/10.1007/s12672-025-04038-1

Article in Press

Non-WNT/non-SHH medulloblastoma in siblings: case report and literature review

Received: 9 June 2025

Accepted: 6 November 2025

Published online: 20 November 2025

Cite this article as: Huang M., Li J., Liu B. *et al.* Non-WNT/non-SHH medulloblastoma in siblings: case report and literature review. *Discov Onc* (2025). https://doi.org/10.1007/s12672-025-04038-1

Meng Huang, Jian Li, Bo Liu, Yuxiang Liao, Bin Wang, Haipeng Liu, Ying Liu & Jie Zhao

Non-WNT/non-SHH medulloblastoma in siblings: case report and literature review

Meng Huang[1*], Jian Li[1*], Bo Liu[1] , Yuxiang Liao[1], Bin Wang[1], Haipeng Liu[1], Ying Liu[1-5], Jie Zhao[1]

Affiliation:

1. Department of Neurosurgery in Xiangya Hospital, Central South University, Changsha, Hunan，410008, China
2. Department of Pathophysiology, School of Basic Medical Sciences, Central South University, Changsha, Hunan 410013, China
3. Sepsis Translational Medicine Key Lab of Hunan Province, Central South University, Changsha, Hunan 410013, China
4. National Medicine Functional Experimental Teaching Center, Changsha, Hunan 410013, China
5. National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China;

*These two authors contributed equally to this study and share the first authorship.

Correspondence:

Jie Zhao
Department of Neurosurgery in Xiangya Hospital, Central South University, 410008, Changsha, China
Steelzj@126.com

Ying Liu
Department of Neurosurgery in Xiangya Hospital, Central South University, 410008, Changsha, China
Department of Pathophysiology, School of Basic Medical Sciences, Central South University, Changsha, Hunan, China
Sepsis Translational Medicine Key Lab of Hunan Province, Central South University, Changsha, Hunan 410013, China
National Medicine Functional Experimental Teaching Center, Changsha, Hunan 410013, China
National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China;
598170861@qq.com

Word Count:
Abstract: 185;

Main Text: 2893;
This manuscript includes 2 table, 3 figures and 4 supporting information files.

Running Title: Non-WNT/non-SHH medulloblastoma in siblings.

Abbreviations:

Abbreviation	Full Name
MBs	medulloblastomas
CNS	central nervous system
WES	Whole exome sequencing
SNVs	Single Nucleotide Variants
CNVs	Copy Number Variants
WNT	Wingless-type
SHH	Sonic Hedgehog
FAP	familial adenomatous polyposis
HRR	homologous recombination repair
FA	Fanconi anemia
NF1	Neurofibromatosis type 1
RAS	Rat sarcoma
MAPK	Mitogen-activated protein kinase
CSF	cerebro-spinal fluid
WHO	World Health Organization
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
MB-G3	mitochondrial subtype MB-G3
ELP1	Elongator complex protein 1
GPR161	G protein-coupled receptor 161
KYAT3	Kynurenine Aminotransferase 3
CBWD6	COBW domain-containing 6
SPATA31A6	SPATA31 subfamily A member 6
OR4F6	Olfactory Receptor family 4 subfamily F member 6
MMR	Mismatch Repair
APC	Adenomatous Polyposis Coli
PALB2	Partner and Localizer of BRCA2
BRCA2	Breast Cancer type 2 susceptibility protein
TP53	Tumor Protein p53

SOX9	SRY-Box Transcription Factor 9
SSTR2	Somatostatin Receptor 2
GFAP	Glial Fibrillary Acidic Protein
EMA	Epithelial Membrane Antigen
NeuN	Neuronal Nuclei
IN1	Integrase Interactor 1
Olig2	Oligodendrocyte Transcription Factor 2
Ki67	Kiel 67 antigen
Syn	Synaptophysin
β-Catenin	Beta-Catenin
YAP1	Yes-Associated Protein 1
GAB1	GRB2-Associated Binding Protein 1
CREBBP	CREB-Binding Protein
EP300	E1A Binding Protein p300
PTEN	Phosphatase and Tensin Homolog
MYC	MYC Proto-Oncogene
MYCN	MYCN Proto-Oncogene
MSH6	MutS Homolog 6
PMS2	Postmeiotic Segregation Increased 2

51

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Xiangya Hospital, Central South University (Approval Number: 2025081132) . All procedures performed in studies involving human participants were in accordance with the ethical standards of the Declaration of Helsinki. Informed consent for genetic analysis and data publication was obtained from both parents of two patients in the study prior to clinical data and sample collection.

Consent for publication

Written informed consents to submit and publish this case report were acquired from both parents of two patients in the study prior to submission.

Availability of data and materials

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive[1] in National Genomics Data Center[2], China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: HRA013031) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Competing interests

The authors have no conflicts of interest to disclose.

Funding

This study was supported by the National Natural Science Foundation of China (grant numbers 82303140，82172147, 81571880, 81373147, 30901555 and 30972870)) and the Natural Science Foundation of Hunan Province (grant nos. 2021JJ30900 and 2016JJ2157, 2025JJ60600).

Authors’ contributions

YL and JZ: Study concept and design; acquisition, analysis, interpretation, and visualization of data; review of the manuscript. MH and LJ: Acquisition, analysis, interpretation, and visualization of data; drafting of the manuscript. BL and YL: Contribution of the patient; analysis of the data. BW and HL: acquisition and interpretation of data. All authors read and approved the final manuscript.

Acknowledgements

We would like to express our gratitude to our patient’s family for their generous donation.

Abstract

Purpose: Medulloblastoma (MB), a malignant cerebellar embryonal tumor, is predominantly sporadic, with rare familial cases linked to germline mutations in *SUFU*, *PTCH1*, *TP53*, and *APC*, primarily driving WNT and SHH molecular subtypes. Familial non-*WNT*/non-*SHH* MBs remain poorly understood, with limited genetic insights.

Methods: This study presents two siblings (11-year-old male, 16-year-old female) with non-*WNT*/non-*SHH* MBs. Whole exome sequencing (WES) was performed on tumor samples (MB1-T, MB2-T), peripheral blood (MB2-PB, parents), and analyzed for somatic/germline variants. Screening criteria excluded intronic/synonymous single nucleotide variants (SNVs) and common alleles.

Results: MB2 harbored somatic chromosome 17q gain (prognostic marker) and two deleterious *NF1* mutations (p.G2785V, p.N2788Y), absent in MB1. Germline analysis identified rare variants in *KYAT3, SPATA31A6*, and *CBWD6*, with potential roles in metabolic reprogramming (*KYAT3*) and genomic instability (*SPATA31A6*). No known MB predisposition syndromes or mutations were detected.

Conclusion: This first report of familial non-*WNT*/non-*SHH* MBs highlights novel somatic (*NF1*) and germline (*KYAT3, SPATA31A6*) variants, suggesting unexplored oncogenic mechanisms. The findings underscore the need for further research to elucidate drivers of non-*WNT*/non-*SHH* MBs and develop targeted therapies. WES proves critical in uncovering genetic underpinnings of rare familial MBs.

Keywords: Familiar medulloblastoma； Germline mutation； Whole exome sequencing, Non-*WNT*/non-*SHH* medulloblastomas.

Introduction

Medulloblastomas(MBs) are the most common malignancies of central nervous system (CNS) for childhood, which are characterized by highly intratumoral heterogeneity, aggressive biological behavior and overall poor outcomes.[3] Based on the recent molecular findings, MBs have been divided into four groups: *WNT*-activated; *SHH*-activated, *TP53*-wildtype; *SHH*-activated, *TP53*-mutant; and non-*WNT*/non-*SHH*.[4] Furthermore, the non-WNT/non-SHH category itself encompasses a spectrum of diseases with considerable clinical and biological heterogeneity. Historically, this category was subdivided into Group 3 and Group 4, which exhibit vast differences in prognosis and underlying biology. Advances in molecular profiling, particularly DNA methylation analysis, have revealed that these groups can be further stratified into at least eight distinct subtypes, each with unique potentially genetic drivers and clinical outcomes[5-7]. However, the underlying mechanism of MBs' genetic pathogenesis remains largely unclear. A large cohort including 1022 patients with MBs identified *APC, BRCA2, PALB2, PTCH1, SUFU* and *TP53* as predisposition genes for MBs, and those germline mutations were estimated to account for only 6% of all MBs, suggesting that the driving factors for the majority of MBs remains unknown. Most MBs arise sporadically, but a few of them can be relevant with certain genetic predisposition syndromes, including Gorlin syndrome (*SUFU* and *PTCH1* mutations), familial adenomatous polyposis (*FAP*) syndrome (*APC* mutations) and Li-Fraumeni syndrome (*TP53* mutations).[3, 8, 9] Beyond the classic syndromes, recent studies have identified novel predisposition genes such as *ELP1*, which is now recognized as a significant contributor to *SHH*-activated medulloblastoma, underscoring the evolving landscape of genetic risk factors for this disease[10, 11]. Notably, most of these genetic variants are involved in the molecular pathogenesis of *SHH* and *WNT* MBs, but not non-*WNT*/ non-*SHH* MBs. This is in accordance with the consensus that non-*WNT*/non-*SHH* MBs frequently harbor rare individual genetic alterations[12, 13].

Here we report two cases of MBs in two siblings (one of each gender). No evidence was found for the diagnosis of those genetic predisposition syndromes mentioned above. Pathological and immunohistochemical analysis confirmed that both cases were classified as non-*WNT*/ non-*SHH* subgroup and histologically classic variant. The whole exome sequencing (WES) were applied for both cases and several potential genomic features that possibly drove the oncogenesis of MBs were identified. We then compared our cases with previously reported cases of familial MBs. To our knowledge, this is the first report in the literature that introduces the familial MBs of non-*WNT*/non-*SHH* with detailed genetic sequencing results.

Methods

DNA extraction and library construction

Genomic DNA was extracted from peripheral blood of the parients and MB-2 patients, and tumors from MB-1 and MB-2 tumors. DNA quantification and integrity were determined by the Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE) and the 1% agarose electrophoresis, respectively.

Human genomic DNA samples were captured using Agilent SureSelect Human All Exon v6 library (Agilent Technologies, USA) following the manufacturer's protocol. Briefly, the genomic DNA was sheared into short fragments using the kit's enzyme. The sheared deoxyribonucleic acid (DNA) was purified and treated with reagents supplied with the kit according to the protocol. Adapters from Agilent were ligated onto the polished ends and the libraries were amplified by polymerase chain reaction (PCR). The amplified libraries were hybridized with the custom probes. The DNA fragments bound with the probes were washed and eluted with the buffer provided in the kit. Then these libraries were sequenced on the Illumina sequencing platform (NovaSeq 6000, Illumina, Inc., San Diego, CA) and 150 bp paired-end reads were generated. The whole exome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

Whole exome sequencing analysis process

The raw data was compiled in fastq format. In order to get high quality reads that can be used for subsequent analysis, the raw reads were pre-processed with fastp (Version: 0.20.0). Firstly, adapter sequences were trimmed. Bases in a sliding widows with average quality value below 20 were also trimmed. Then reads with ambiguous bases or shorter than 75 bp were also removed.

Clean reads were aligned to the reference human genome (GRCh37) utilizing the BWA (Version: 0.7.17). The mapped reads were sorted and indexed by using SAMtools (Version: 1.9). The read depth of were 267x, 271x, 267x, 451x and 284x for sample MB2-T, MB1-T, MB2-PB, F-PB and M-PB respectively. The GATK (Version: 4.1.9.0) was used for recalibration of the base quality score and for single nucleotide polymorphism (SNP) and insertion/deletion (INDELs) realignment. Many annotation databases, such as Refseq, 1000 Genomes, the Catalogue of Somatic Mutations in Cancer (COSMIC), OMIM, were referred to during SNP&INDEL calling and annotated using ANNOVAR. MuTect (Version: 2.0) software was used to find somatic SNV/INDEL sites, and ANNOVAR software was used for annotation. The CNV analysis was performed with CNVkit software and human genome GRCh37.p13 was applied as reference. Control-FREEC (Version: 11.3) software was used to detect somatic CNV on tumor and normal paired samples with default parameters. Lumpy (Version: 0.2.13) software was used to detect somatic SV for tumor and normal paired samples. The detected genomic variation information was visualized using the Circos diagram.

Case presentation

The first case (MB1) is a 11-year-old male patient with a half-a-year history of headache. CT scan showed a foci with slightly higher density in the tentorium area. Following magnetic resonance imaging revealed a 34mm * 32mm mass in the fourth ventricle with obstructive hydrocephalus (Figure 1. The tumor was resected as completely as possible and the postoperative pathology/ immunohistochemistry staining examination confirmed the diagnosis of medulloblastoma with classic histological variant and non-WNT/non-SHH subgroup. The result of immunohistochemistry was shown in Table 1. The patient received magnetic resonance imaging of full-length spinal cord at the time when he was diagnosed and no aberrant signal was detected. But the thinprep cytologic test of cerebro-spinal fluid (CSF) showed the presence of red blood cells, lymphocytes/monocytes and aberrant cell clusters with round, large cellular body and round/quasi-circular/irregular, large nuclei, indicating the possibility of CSF metastasis of MB tumor cells. His condition deteriorated rapidly post-surgery with widespread metastasis, rendering him unable to tolerate intensive chemotherapy. The patient died six months later due to a multiple metastasis in the spinal cord.

The second case (MB2) is a 16-year-old female patient who is the older sister of the first case. Two years after the first case, she received magnetic resonance imaging due to two times of headache and vomiting, and the result showed both lesions in the suprasellar region and fourth ventricle with obstructive hydrocephalus and interstitial cerebral edema, suggesting a likelihood of ectopic germinoma or medulloblastoma (Figure 1. After the discussion of our medical detection team, surgery was performed to remove the lesion in the fourth ventricle to clarify the pathological diagnosis and relieve symptoms of hydrocephalus. The postoperative pathological diagnosis was non-*WNT*/non-*SHH* MB with the classic histological variant. The result of immunohistochemistry was shown in Table 1. She developed a severe infection after undergoing the first course of standardized chemotherapy, which even led to septic shock, so the regimen of radiotherapy and Bevacizumab was decided upon by a multidisciplinary team based on an individualized assessment of her condition at that time.

No familial history of malignancy or hereditary disease among first- and second-degree relatives was found for these two siblings. Due to the rarity of familial MBs of non-*WNT*/non-*SHH* subgroup, WES was suggested to the patients to reveal the potential driving factor for oncogenesis. After written informed consent was obtained from both patients, tumor sample of case 1 (MB1-T), tumor sample and peripheral blood sample of case 2 (MB2-T and MB2-PB), peripheral blood sample of father and mother (F-PB and M-PB) were sent for the WES, and the analysis and genetic variants screening were

conducted with general protocol.

The CNV profiles of both parents' peripheral blood samples were first compared with human genomic reference GRCh37.p13 and nothing abnormal was identified, indicating that germline pathogenic CNV was absent in this family (Supplementary Figure 1). Given the unavailability of a matched germline control (e.g., peripheral blood) from patient MB1, somatic copy number alteration (CNA) analysis could not be performed to distinguish somatic events from inherited germline CNVs. Nevertheless, we conducted a preliminary comparison of the MB1 tumor WES data against the human reference genome(Figure 2A). This analysis revealed several chromosomal imbalances, including gains at 1q and 7q, as well as losses at 3q and 10q (Figure 2B).

The somatic variants of MB2 were then analyzed (Fig 3A). Somatic chromosome gain of 1q,17q and loss of 1p, 8p, 14q and 16q were identified in MB2 (Fig 3B). Chromosome 17q gain was one of the most powerful prognostic markers of MBs[14, 15], which involves several MB-related genes such as *SOX9* and *SSTR2*[16-18]. Besides, an overall of 475 non-intronic and non-synonymous somatic SNVs were identified in MB2. Notably, two mutations of gene *NF1* were revealed: NF1: NM_000267.3:exon57:c.G8354T:p.G2785V,NF1: NM_001042492.3:exon58:c.G8417T:p.G2806V and NF1:NM_000267.3:exon57:c.A8362T:p.N2788Y,NF1:NM_001042492.3:exon58:c.A8425T:p.N2809Y. The variant allele frequencies (VAFs) for these two mutations were about 5%. Both *NF1* mutations are labelled as deleterious in SIFT database and disease-causing in MutationTaster database. However, these two mutations were not detected in the MB1 case.

We next looked into the potentially recessive genetic mutations due to the rare familial history. A overall of 673 genetic variants were detected, and a primary screening were conducted by the following criteria: 1) all intronic SNVs were excluded, 2) all synonymous SNVs were excluded; 3) SNVs with alternative allele frequency above 1% in the 1000 Genomes Project and Exome Aggregation consortium were excluded. This led to a short list of only 9 genetic variants including genes such as *KYAT3, SPATA31A6, CBWD6* and *ANO5* (Supplementary table 1). A similar screening were performed for potentially de novo genetic mutations and 6 genetic variants were identified including involved genes such as *SPATA31A6, CBWD6* and *OR4F6* (Supplementary table 2). The summary of those genes and their potentially involved diseases are listed in Table 2. We evaluated the expression levels of these genes using medulloblastoma transcriptome sequencing data from public databases (GSE85217). Among them, genes *CBWD6* and *SPATA31A6* were not detectable, while the expression levels of the other genes exhibited molecular subtype-

specific patterns (Supplementary Figure 2).

Discussion and conclusions

Familial medulloblastoma is a rare but clinically significant entity that provides insights into the genetic and molecular mechanisms of tumorigenesis. Cornelia et al. well addressed previously published cases of familial medulloblastomas[19]. However, most of the reports included lack the results of molecular testing. Recent studies revealed that familial medulloblastomas are often linked to inherited genetic syndromes, including Gorlin syndrome (*PTCH1* and *SUFU* mutations)[20-22], Li-Fraumeni syndrome (*TP53* mutations)[23-27], and familial FAP syndrome (APC mutations)[28]. These conditions follow an autosomal dominant inheritance pattern, and case reports frequently describe families with multiple members affected by both medulloblastoma and other malignancies. Other syndromes, such as Beckwith-Wiedemann syndrome (epimutations)[29, 30], Cowden syndrome (*PTEN* mutations)[31] and Rubinstein-Taybi syndrome (*CREBBP* and *EP300* mutations)[32, 33], have been less frequently associated but still highlight the genetic predisposition to medulloblastoma. Additionally, germline mutations in *BRCA2* and *PALB2*, key genes involved in the homologous recombination repair (HRR) pathway, have been implicated in the development of medulloblastoma, often in the context of genetic predisposition syndromes such as Fanconi anemia (FA)[23, 34]. Recent studies have also identified *ELP1* and *GPR161* as potential genetic contributors to familial medulloblastoma, particularly in the SHH subgroup. *ELP1* loss-of-function variants are associated with a 3.2% incidence of medulloblastoma[11]. Similarly, *GPR161* variants, though rarer, have been implicated in medulloblastoma pathogenesis[35, 36]. Notably, most of genes mentioned above were not involved in non-*WNT*/non-*SHH* MBs[37]. Meanwhile, these genes were not detected in two cases included in this study either, suggesting more unknown oncogenic mechanisms of those patients.

Our whole exome sequencing data revealed two somatic *NF1* mutations in MB2. Neurofibromatosis type 1 (NF1), a genetic disorder caused by mutations in the *NF1* gene, is known for its association with multiple neurofibromas, café-au-lait spots, and an increased risk of various malignancies[38]. Although less common in NF1 patients, medulloblastomas have been documented in several case reports[39, 40]. The rarity of medulloblastoma in NF1 patients suggests that *NF1* gene mutations do not significantly predispose individuals to this type of tumor directly. However, *NF1* encodes neurofibromin, which plays as a key negative regulator of the RAS/MAPK signaling pathway. *NF1* mutations have also been implicated in other cancers, including breast cancer, melanoma, and lung cancer, where aberrant RAS signaling plays a critical oncogenic role[41]. Given its central role in RAS regulation, the NF1 mutations

might till have played a promotive role in the tumorigenesis and progression of MB2.

The analysis of germline mutations included MB1 and MB2 revealed several previously less-discussed genes, such as *KYAT3, SPATA31A6* , *CBWD6 and ANO5*. The *KYAT3* (Kynurenine Aminotransferase 3) gene, involved in the kynurenine pathway, may play a role in medulloblastoma pathogenesis. *KYAT3* regulates the conversion of kynurenine to kynurenic acid, a metabolite that modulates immune responses and glutamatergic signaling[42]. Dysregulation of the kynurenine pathway has been linked to immune evasion in tumors by suppressing T-cell activation[43, 44]. Additionally, altered amino acid metabolism is a hallmark of *MYC*-amplified medulloblastoma subtypes, suggesting a potential role for *KYAT3* in metabolic reprogramming[45-47]. Future studies should explore *KYAT3* expression in medulloblastoma and assess its potential as an oncogenic driver therapeutic target.

Although no direct evidence links *SPATA31A6* mutations to medulloblastoma, its potential role in DNA repair and genomic stability suggests a possible connection. *SPATA31A6* belongs to the SPATA31 gene family, which has been implicated in UV-induced DNA damage repair and aging[48]. Given that genomic instability is a key driver of medulloblastoma, mutations in *SPATA31A6* could contribute to tumorigenesis by impairing DNA repair pathways, leading to an accumulation of oncogenic mutations. If SPATA31A6 mutations were causally involved in tumorigenesis through impairing DNA damage repair, one might hypothesize an associated mutational signature, such as a UV signature or signature of defective DNA repair, and a higher overall tumor mutation burden (TMB). Future studies with whole-genome sequencing could investigate the mutational signatures in tumors arising in individuals with such variants to test this hypothesis.

As a member of the *COBW* domain-containing family, *CBWD6* is a less characterized gene. There is currently no direct evidence linking *CBWD6* to medulloblastoma. Future studies should investigate *CBWD6* expression levels, genetic alterations, and functional roles in medulloblastoma to determine its potential involvement in tumorigenesis.

Although the current study identified a rare variant in ANO5, its direct role in medulloblastoma tumorigenesis remains unclear and has not been previously established in the literature. ANO5 encodes anoctamin-5, a protein that functions as a calcium-activated chloride channel (CaCC) involved in membrane repair and phospholipid scrambling. While ANO5 mutations are primarily associated with gnathodiaphyseal dysplasia and limb-girdle muscular dystrophy[49, 50], emerging evidence suggests that ion channels, including chloride channels, may contribute to cancer progression by affecting

cell volume regulation, migration, and proliferation[51]. Dysregulation of calcium and chloride signaling has been implicated in various cancers by modulating apoptosis resistance and metastatic potential[52-54]. Although no direct link between ANO5 and medulloblastoma has been reported, it is plausible that deleterious mutations could disrupt membrane stability and ion homeostasis, potentially influencing neoplastic transformation or tumor cell survival in the central nervous system. Further functional studies are needed to elucidate whether ANO5 variants play a cooperative or modifying role in medulloblastoma development, particularly in the context of familial cases.

The lack of a shared, obvious pathogenic germline variant in these siblings suggests several possibilities: 1) a shared, but undetected, low-penetrance genetic risk factor or a combination of such factors (oligogenic inheritance); 2) a shared environmental exposure acting on a permissive but complex genetic background; or 3) an inherited variant in a non-coding regulatory region not effectively covered by WES. The latter possibility highlights the potential utility of whole-genome sequencing (WGS) in such unexplained familial cases. Future studies of such rare familial cases could benefit from WGS, which might uncover structural variants, mutations in non-coding regions, or complex rearrangements missed by WES.

One limitation of our study is the fact that patient1 MB1 passed away when we initiated this study, therefore we were unable to obtain a matched germline (e.g., peripheral blood) patient MB1 and we could not perform CNV or somatic variation analysis for this case to revealed shared somatic CNVs between tumors. Another significant limitation of this study is the lack of DNA methylation profiling or transcriptomic analysis, which precludes the precise molecular sub-classification of these non-*WNT*/non-*SHH* tumors into the recognized Group 3 or Group 4 subtypes and their further subdivisions. Determining whether these siblings shared the same molecular subgroup would have been highly informative.

The genetic mutations identified in this study have not been previously reported to be associated with the development of medulloblastoma. Therefore, subsequent research should further investigate the potential mechanisms by which these genes drive tumorigenesis and develop novel targeted therapeutic strategies based on these findings. Additionally, elucidating the oncogenic potential of these genetic mutations will facilitate the implementation of genetic screening programs for high-risk populations.

Familial medulloblastoma represents a unique subset of pediatric brain tumors with significant genetic implications. While most reported cases are linked to known hereditary cancer syndromes, some remain unexplained, warranting further genetic investigations. The integration of WES data from

new cases, such as the one presented here, will enhance our understanding of this disease and pave the way for improved diagnostic and therapeutic strategies. Future research should focus on expanding the genetic landscape of familial MB and exploring its implications for personalized medicine. The addition of our newly identified cases contributes to the growing body of evidence, underscoring the need for continued research in familial medulloblastoma predisposition and management.

Case No.	Age	Gender	Pathology	Molecular subgroup	Immunohistochemistry results
1	11	Male	Classic	non-WNT/non-SHH	GAB1(-) ， YAP1(-) ， β-Catenin(-) ， P53(wildtype) ， Syn(+) ， Ki67(10%+) ， NeuN(-) ， IN1(+) ， GFAP(-) ， 0lig2(-), EMA(-)
2	16	Female	Classic	non-WNT/non-SHH	GAB1(-) ， YAP1(-) ， β-Catenin(cytoplasm +) ， P53(wildtype) ， Syn(+) ， Ki67(20%+) ， NeuN(+) ， IN1(+) ， GFAP(-) ， 0lig2(scattered+)

Table 1. Clinical manifestation of two cases of MBs.

Gene Symbol	Full Name	Chromosomal Location	Encoded Protein	Primary Functions and Pathways Involved	Diseases/Cancers with Literature Reports (if available)
NF1	Neurofibromin 1	17q11.2	Neurofibromin	Functions as a RAS GTPase-activating protein (GAP), negatively regulating the RAS/MAPK signaling pathway; controls cell growth and differentiation.	Neurofibromatosis type 1 (NF1); associated with increased risk of various benign and malignant tumors, including optic pathway glioma and malignant peripheral nerve sheath tumor (MPNST)[38].
KYAT3	Kynurenine Aminotransferase 3	1p22.2	Kynurenine aminotransferase 3	Involved in the tryptophan metabolic pathway (kynurenin	Unclear

				e pathway); catalyzes the conversion of kynurenine to kynurenic acid.	
SPATA31A6	SPATA31 Subfamily A Member 6	9p11.2	Spermatogenesis-Associated Protein 31A6	Predicted to be involved in cell differentiation and spermatogenesis. Potentially involved in the response to UV-induced DNA damage repair.	Unclear
CBWD6	COBW Domain Containing 6; Also known as : ZNG1F	9p11.2	Cobalamin synthase W domain-containing protein 6; Zinc-Regulated GTPase Metalloprotein Activator 1F.	Predicted to enable GTP binding activity; hydrolase activity; and metal ion binding activity.	May be associated with intracranial aneurysms[55]; No clear cancer associations have been reported.
ANO5	Anoctamin 5	11p14.3	Anoctamin-5	Encodes a transmembrane protein of the anoctamin family, likely	Associated with gnathodiaphyseal dysplasia[50], Muscular Dystrophy, Limb-Girdle, Autosomal

				functioning as a calcium-activated chloride channel.	Recessive 12[49]; Involved in gastric cancer[54], prostate cancer[53], and thyroid cancer[52].
OR4F6	Olfactory Receptor Family 4 Subfamily F Member 6	15q26.3	Olfactory receptor 4F6	Mediates olfactory signal transduction; exhibits G protein-coupled receptor (GPCR) activity.	Possibily related to early-onset type 2 diabetes mellitus, metabolic syndrome, or hyperuricemia[56]; no clear association with tumors has been reported.
FGF7P6	Fibroblast Growth Factor 7 Pseudogene 6 (Note: a pseudogene)	9q13	Generally does not encode a functional protein	Pseudogenes are often evolutionary remnants; may be involved in gene regulation but typically do not encode functional proteins.	Unclear
GGT3P	Putative gamma-glutamyltransp eptidase 3 (Note: likely pseudogene)	22q11.21	Putative gamma-glutamyltransp eptidase 3	May be involved in extracellular glutathione (GSH) breakdown; catalyzes	Unclear

					the transfer of the glutamyl group of glutathione to amino acid and dipeptide acceptors.

Table 2. Summary of genes identified in WES analysis.

Figures

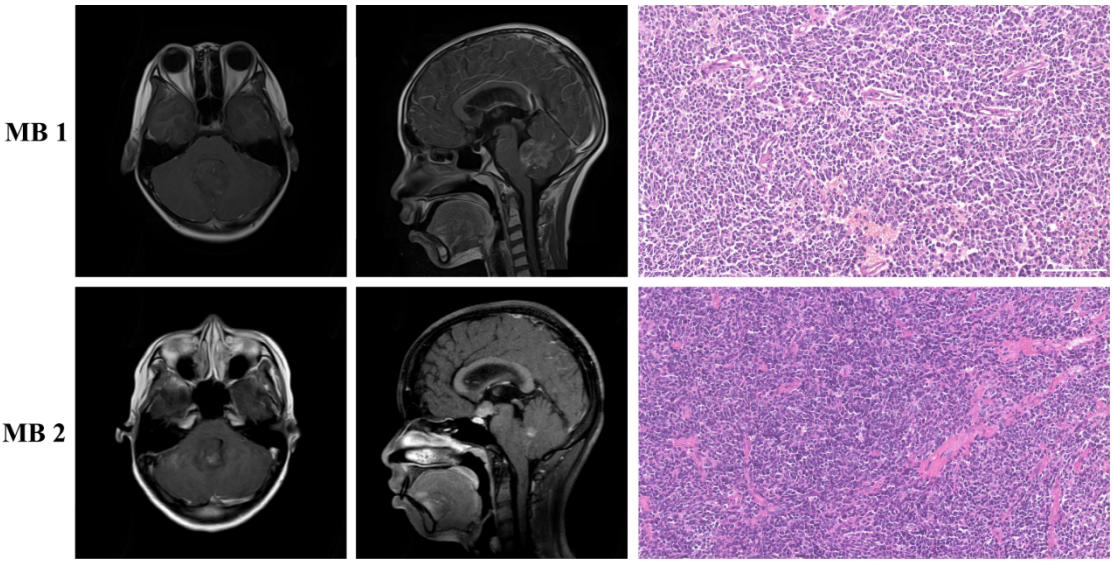

Figure 1. Representative images of magnetic resonance imaging and histology of two medulloblastoma cases. Scare bar, 100μm.

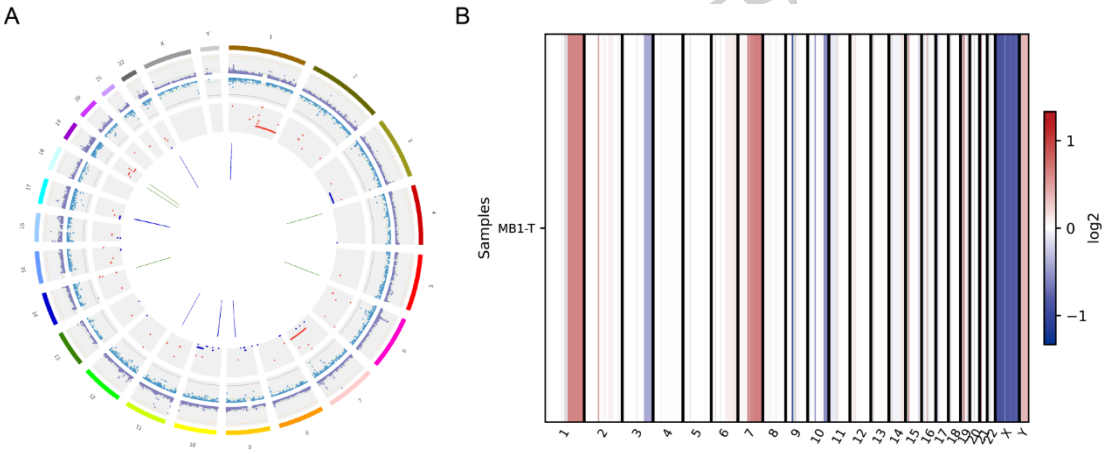

Figure 2. WES profiles of MB1-T. A. Circos plot of all variants of MB1. B. CNVs identified of MB1.

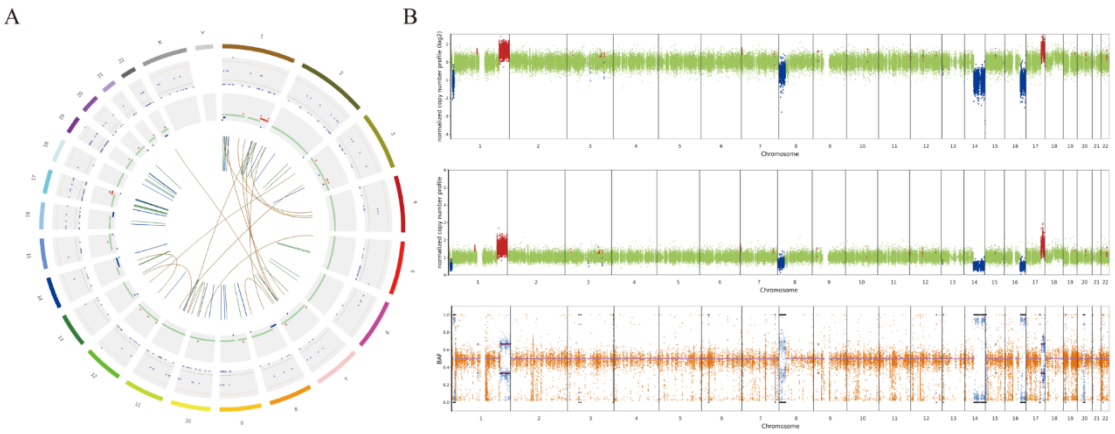

Figure 3. Somatic variants identified by WES of MB2. A. Circos plot of all somatic variants of MB2. B. Somatic CNVs identified of MB2.

Supplementary Table 1: Recessive germline genetic mutations identified for MB1 and MB2 patients. Detailed header description are listed.
Supplementary Table 2: De novo germline genetic mutations identified for MB1 and MB2 patients. Detailed header description are listed.

Supplementary Figure 1: CNV profiles of samples of two parents
Supplementary Figure 2: Different expression patterns of genes identified. The sequencing data was obtained from GSE database (GSE85217).

Reference:

[1] Chen T, Chen X, Zhang S, Zhu J, Tang B, Wang A, et al. The Genome Sequence Archive Family: Toward Explosive Data Growth and Diverse Data Types. Genomics Proteomics Bioinformatics. 2021;19(4):578-83.
[2] Members C-N, Partners. Database Resources of the National Genomics Data Center, China National Center for Bioinformation in 2022. Nucleic Acids Res. 2022;50(D1):D27-D38.
[3] Northcott PA, Robinson GW, Kratz CP, Mabbott DJ, Pomeroy SL, Clifford SC, et al. Medulloblastoma. Nat Rev Dis Primers. 2019;5(1):11.
[4] Horbinski C, Berger T, Packer RJ, Wen PY. Clinical implications of the 2021 edition of the WHO classification of central nervous system tumours. Nature reviews Neurology. 2022;18(9):515-29.
[5] Foti A, Allia F, Briglia M, Malaguarnera R, Tamburrini G, Cecconi F, et al. Medulloblastoma: Molecular Targets and Innovative Theranostic Approaches. Pharmaceutics. 2025;17(6).
[6] Kiang KM, Wong YKH, Sengupta S, Roussel MF, Lu QR. Developmental Origins and Oncogenesis in Medulloblastoma. Annu Rev Neurosci. 2025;48(1):85-102.
[7] Williamson D, Schwalbe EC, Hicks D, Aldinger KA, Lindsey JC, Crosier S, et al. Medulloblastoma group 3 and 4 tumors comprise a clinically and biologically significant expression continuum reflecting human cerebellar development. Cell Rep. 2022;40(5):111162.
[8] Chen Y, Zhang H, Zhao Y, Ma J. Congenital medulloblastoma in two brothers with SUFU-mutated Gorlin-Goltz syndrome: Case reports and literature review. Front Oncol. 2022;12:988798.
[9] Massimino M, Signoroni S, Boschetti L, Chiapparini L, Erbetta A, Biassoni V, et al. Medulloblastoma and familial adenomatous polyposis: Good prognosis and good quality of life in the long-term? Pediatr Blood Cancer. 2021;68(4):e28912.
[10]Farouk Sait S, Walsh MF, Karajannis MA. Genetic syndromes predisposing to

pediatric brain tumors. Neurooncol Pract. 2021;8(4):375-90.
[11]Waszak SM, Robinson GW, Gudenas BL, Smith KS, Forget A, Kojic M, et al. Germline Elongator mutations in Sonic Hedgehog medulloblastoma. Nature. 2020;580(7803):396-401.
[12]Liu X, Ding C, Tan W, Zhang A. Medulloblastoma: Molecular understanding, treatment evolution, and new developments. Pharmacology & therapeutics. 2020;210:107516.
[13]Jackson K, Packer RJ. Recent Advances in Pediatric Medulloblastoma. Curr Neurol Neurosci Rep. 2023;23(12):841-8.
[14]Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, et al. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27(10):1627-36.
[15]Korshunov A, Remke M, Werft W, Benner A, Ryzhova M, Witt H, et al. Adult and pediatric medulloblastomas are genetically distinct and require different algorithms for molecular risk stratification. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(18):3054-60.
[16]Guyotat J, Champier J, Pierre GS, Jouvet A, Bret P, Brisson C, et al. Differential expression of somatostatin receptors in medulloblastoma. Journal of neuro-oncology. 2001;51(2):93-103.
[17]Adolphe C, Millar A, Kojic M, Barkauskas DS, Sundstrom A, Swartling FJ, et al. SOX9 Defines Distinct Populations of Cells in SHH Medulloblastoma but Is Not Required for Math1-Driven Tumor Formation. Molecular cancer research : MCR. 2021;19(11):1831-9.
[18]Li Q, Jia Y, Tang B, Yang H, Yang Q, Luo X, et al. Mitochondrial subtype MB-G3 contains potential novel biomarkers and therapeutic targets associated with prognosis of medulloblastoma. Biomarkers. 2023;28(7):643-51.
[19]von Koch CS, Gulati M, Aldape K, Berger MS. Familial medulloblastoma: case report of one family and review of the literature. Neurosurgery. 2002;51(1):227-33; discussion 33.
[20]Evans DG, Farndon PA, Burnell LD, Gattamaneni HR, Birch JM. The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. Br J Cancer. 1991;64(5):959-61.
[21]Amlashi SF, Riffaud L, Brassier G, Morandi X. Nevoid basal cell carcinoma syndrome: relation with desmoplastic medulloblastoma in infancy. A population-based study and review of the literature. Cancer. 2003;98(3):618-24.
[22]Garre ML, Cama A, Bagnasco F, Morana G, Giangaspero F, Brisigotti M, et al. Medulloblastoma variants: age-dependent occurrence and relation to Gorlin syndrome--a new clinical perspective. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(7):2463-71.
[23]Waszak SM, Northcott PA, Buchhalter I, Robinson GW, Sutter C, Groebner S, et al. Spectrum and prevalence of genetic predisposition in medulloblastoma: a retrospective genetic study and prospective validation in a clinical trial cohort. The Lancet Oncology. 2018;19(6):785-98.

[24]Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJ, Martin DC, et al. Subgroup-specific prognostic implications of TP53 mutation in medulloblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(23):2927-35.
[25]Villani A, Shore A, Wasserman JD, Stephens D, Kim RH, Druker H, et al. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. The Lancet Oncology. 2016;17(9):1295-305.
[26]Tabori U, Baskin B, Shago M, Alon N, Taylor MD, Ray PN, et al. Universal poor survival in children with medulloblastoma harboring somatic TP53 mutations. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(8):1345-50.
[27]Northcott PA, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T, et al. The whole-genome landscape of medulloblastoma subtypes. Nature. 2017;547(7663):311-7.
[28]Georgescu MM, Whipple SG, Notarianni CM. Novel neoplasms associated with syndromic pediatric medulloblastoma: integrated pathway delineation for personalized therapy. Cell Commun Signal. 2022;20(1):123.
[29]Silva FLT, Ruas JS, Euzebio MF, Hoffmann IL, Junqueira T, Tedeschi H, et al. 11p15 Epimutations in Pediatric Embryonic Tumors: Insights from a Methylome Analysis. Cancers (Basel). 2023;15(17).
[30]Olson JM, Breslow NE, Barce J. Cancer in twins of Wilms tumor patients. Am J Med Genet. 1993;47(1):91-4.
[31]Albrecht S, Miedzybrodzki B, Palma L, Nguyen VH, Dudley RWR, Pietsch T, et al. Medulloblastoma and Cowden syndrome: Further evidence of an association. Free Neuropathol. 2022;3.
[32]Bourdeaut F, Miquel C, Richer W, Grill J, Zerah M, Grison C, et al. Rubinstein-Taybi syndrome predisposing to non-WNT, non-SHH, group 3 medulloblastoma. Pediatr Blood Cancer. 2014;61(2):383-6.
[33]Merk DJ, Ohli J, Merk ND, Thatikonda V, Morrissy S, Schoof M, et al. Opposing Effects of CREBBP Mutations Govern the Phenotype of Rubinstein-Taybi Syndrome and Adult SHH Medulloblastoma. Dev Cell. 2018;44(6):709-24 e6.
[34]Prakash R, Zhang Y, Feng W, Jasin M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol. 2015;7(4):a016600.
[35]Begemann M, Waszak SM, Robinson GW, Jager N, Sharma T, Knopp C, et al. Germline GPR161 Mutations Predispose to Pediatric Medulloblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020;38(1):43-50.
[36]Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ, et al. The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. Cell. 2013;152(1-2):210-23.
[37]Carta R, Del Baldo G, Miele E, Po A, Besharat ZM, Nazio F, et al. Cancer Predisposition Syndromes and Medulloblastoma in the Molecular Era. Front Oncol.

2020;10:566822.
[38]Campian J, Gutmann DH. CNS Tumors in Neurofibromatosis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35(21):2378-85.
[39]Ranalli M, Boni A, Caroleo AM, Del Baldo G, Rinelli M, Agolini E, et al. Molecular Characterization of Medulloblastoma in a Patient with Neurofibromatosis Type 1: Case Report and Literature Review. Diagnostics (Basel). 2021;11(4).
[40]Pascual-Castroviejo I, Pascual-Pascual SI, Viano J, Carceller F, Gutierrez-Molina M, Morales C, et al. Posterior fossa tumors in children with neurofibromatosis type 1 (NF1). Childs Nerv Syst. 2010;26(11):1599-603.
[41]Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. Lancet Neurol. 2014;13(8):834-43.
[42]Abd El-Fattah EE. IDO/kynurenine pathway in cancer: possible therapeutic approaches. J Transl Med. 2022;20(1):347.
[43]Ala M. The footprint of kynurenine pathway in every cancer: a new target for chemotherapy. Eur J Pharmacol. 2021;896:173921.
[44]Gouasmi R, Ferraro-Peyret C, Nancey S, Coste I, Renno T, Chaveroux C, et al. The Kynurenine Pathway and Cancer: Why Keep It Simple When You Can Make It Complicated. Cancers (Basel). 2022;14(11).
[45]Pham K, Hanaford AR, Poore BA, Maxwell MJ, Sweeney H, Parthasarathy A, et al. Comprehensive Metabolic Profiling of MYC-Amplified Medulloblastoma Tumors Reveals Key Dependencies on Amino Acid, Tricarboxylic Acid and Hexosamine Pathways. Cancers (Basel). 2022;14(5).
[46]Wolin AR, Vincent MY, Hotz T, Purdy SC, Rosenbaum SR, Hughes CJ, et al. EYA2 tyrosine phosphatase inhibition reduces MYC and prevents medulloblastoma progression. Neuro-oncology. 2023;25(12):2287-301.
[47]Adiamah M, Poole B, Lindsey JC, Kohe S, Morcavallo A, Burte F, et al. MYC-dependent upregulation of the de novo serine and glycine synthesis pathway is a targetable metabolic vulnerability in group 3 medulloblastoma. Neuro-oncology. 2025;27(1):237-53.
[48]Bekpen C, Kunzel S, Xie C, Eaaswarkhanth M, Lin YL, Gokcumen O, et al. Segmental duplications and evolutionary acquisition of UV damage response in the SPATA31 gene family of primates and humans. BMC Genomics. 2017;18(1):222.
[49]Topf A, Johnson K, Bates A, Phillips L, Chao KR, England EM, et al. Sequential targeted exome sequencing of 1001 patients affected by unexplained limb-girdle weakness. Genet Med. 2020;22(9):1478-88.
[50]Bolduc V, Marlow G, Boycott KM, Saleki K, Inoue H, Kroon J, et al. Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 cause proximal LGMD2L and distal MMD3 muscular dystrophies. Am J Hum Genet. 2010;86(2):213-21.
[51]Kunzelmann K. Ion channels and cancer. J Membr Biol. 2005;205(3):159-73.
[52]Chang Z, Cai C, Han D, Gao Y, Li Q, Feng L, et al. Anoctamin5 regulates cell migration and invasion in thyroid cancer. International journal of oncology. 2017;51(4):1311-9.

[53]Yu CC, Chen LC, Huang CY, Lin VC, Lu TL, Lee CH, et al. Genetic association analysis identifies a role for ANO5 in prostate cancer progression. Cancer medicine. 2020;9(7):2372-8.

[54]Guan E, Liu H, Xu N. Lidocaine Suppresses Gastric Cancer Development Through Circ_ANO5/miR-21-5p/LIFR Axis. Dig Dis Sci. 2022;67(6):2244-56.

[55]Tutino VM, Lu Y, Ishii D, Poppenberg KE, Rajabzadeh-Oghaz H, Siddiqui AH, et al. Aberrant Whole Blood Gene Expression in the Lumen of Human Intracranial Aneurysms. Diagnostics (Basel). 2021;11(8).

[56]Yamada Y, Kato K, Oguri M, Horibe H, Fujimaki T, Yasukochi Y, et al. Identification of four genes as novel susceptibility loci for early-onset type 2 diabetes mellitus, metabolic syndrome, or hyperuricemia. Biomed Rep. 2018;9(1):21-36.