# CASE REPORT

### Novel PHOX2B germline mutation in childhood medulloblastoma: a case report

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**Abstract**

**Background:** Medulloblastoma is an aggressive brain tumor mostly found in children, few studies on pathogenic germline mutations predisposing this disease was reported.

**Case presentation:** We present an 11-year-old male with medulloblastoma, who harbors a de novo PHOX2B germline mutation as detected by whole exome sequencing (WES). Family history was negative. Sanger sequencing confirmed this mutation in peripheral blood, hair bulbs, urine and saliva. Identification of novel germline mutations is beneficial for childhood cancer screening.

**Conclusions:** This case revealed a de novo PHOX2B germline mutation as a potential cause of medulloblastoma in a child and suggests familial germline variant screening is useful when an affected family is considering having a second child.

**Keywords:** Medulloblastoma, PHOX2B, Germline mutation, Whole exome sequencing, Cancer screening

**Introduction**

Medulloblastoma is a malignant tumor of the cerebellum that is most common in childhood, characterized by highly malignant manifestations including rapid tumor growth, high recurrence rate, and poor overall survival [1]. Large-scale genetic studies have revealed somatic and germline mutations that associated with the disease. One genetic study showed that KBTBD4 and PRDM6 are candidate driver mutations in medulloblastoma [2]. In addition, six germline mutations: APC, BRCA2, PALB2, PTCH1, SUFU, and TP53, were reported to be responsible for 6% of medulloblastoma cases [3]. However, these mutations may not be able to fully explain the susceptibility and pathogenesis of a sporadic case.

PHOX2B encodes neuroblastoma Phox (paired-like homeobox 2B) protein, which plays a role in neuron development and involves in the determination of the neurotransmitter phenotype. It is reported to be associated with congenital central hypoventilation syndrome [4] and hereditary neuroblastic tumours [5]. The pathogenic roles of PHOX2B mutations have been published in the ClinVar database, but few reports exist on de novo germline mutations associated with childhood medulloblastoma development. By using whole exome sequencing (WES, the NovaSeq 6000 Sequencing System, Illumina) technology and Sanger sequencing validation, we report the case of a child with a de novo c.765_779 deletion of PHOX2B as a contributor to the risk of medulloblastoma.
**Methods and results**

**Case descriptions**

An 11-year-old male patient who had an accidental fall in December 2018 with no other past medical history was seen in our hospital. Subsequent head and whole spinal cord MRI showed lesions in the fourth ventricle, suggesting a likelihood of medulloblastoma (Fig. 1a). On 2019-01-10, following general anesthesia, a cranial fossa craniotomy, cerebellar tumor resection, dural repair, and decompressive craniectomy were performed. After the surgical treatment and five cycles of temozolomide, the patient is stable. Postoperative pathology diagnosis was cerebellum medulloblastoma (WHO-IV). Immunohistochemistry showed Vimentin (−), CK (−), GFAP (−), S100 (+/−), Ki67 (30% +), P53 (−), CD99 (−), CD56 (+), SYN (+), and NSE (+) (Fig. 1b).

The patient’s parents were concerned a second child might be affected. Therefore, genetic testing was done in which three mutations were detected, including a c.505A > G point mutation in the MSH2 gene (NM_000251 transcript), a c.6139A > G point mutation in the MED12 gene (NM_005120 transcript) and a c.765_779del deletion mutation in the PHOX2B gene (NM_003924 transcript). The c.505A > G point mutation and the c.765_779del deletion were heterozygous mutations, while the c.6139A > G point mutation was a homozygous mutation. According to the ClinVar database, the c.505A > G point mutation in the MSH2 gene is a possible benign variation (Likely benign), the c.6139A > G point mutation in MED12 is of unknown clinical significance, and the c.765_779del deletion mutation of PHOX2B is Benign/Likely benign. Further

![Fig. 1](image-url) The discovery of a novel PHOX2B mutation. a MRI imaging indicating a 30 mm X 30 mm X 21 mm solid tumor at the fourth ventricle; b Pathological findings of surgical tissue; c Electropherogram showing the c.765_779del mutation in PHOX2B. Other electropherogram images across tissues are list in supplementary Table 1.
familial genetic testing showed that the c.505A > G point mutation of MSH2 and the c.6139A > G point mutation of MED12 were inherited from his mother. Of note, the c.765_779del deletion mutation of PHOX2B was a de novo mutation (Fig. 1c). These germline mutations were confirmed by Sanger sequencing on samples obtained from patient’s peripheral blood, saliva, hair, and urine. We also conducted DNA paternity testing to confirm that the parents are the patient’s biological parents (Table 1, supplementary Table 1).

Sample pre-processing
DNA was extracted from 1 ml of peripheral blood by a Blood genomic DNA Mini Kit (CW2087, Cwbio, China), at least 5 hair bulb and 35 ml urine by the universal genomic DNA Kit (CW2298, Cwbio, China), and 0.8 ml saliva by the CW2655 kit (CW2655, Cwbio, China), according to the manufacturer’s instructions. The extracted DNA was dissolved in 100 μl TE buffer, quantified using a NanoDrop spectrophotometer and stored at –80 °C until use. The Medical Ethics Committee of the Maoming People’s Hospital reviewed and approved this study. Both parents and the patient signed an informed consent. No personal information will be disclosed in this study.

PCR amplification and sanger sequencing
DNA was amplified using specific primers listed below (Table 2). PCR amplification was performed using the following cycle conditions: pre-denaturing at 95 °C for 1 min; 45 cycles consisting of 95 °C for 45 s, 57 °C for 45 s, 68 °C for 1 min; and final extension at 68 °C for 3 min. The PCR products were analysed on a 1% agarose gel. Sequences reactions were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) following the manufacturer’s instructions. The extracted DNA was dissolved in 100 μl TE buffer, quantified using a NanoDrop spectrophotometer and stored at –80 °C until use. The Medical Ethics Committee of the Maoming People’s Hospital reviewed and approved this study. Both parents and the patient signed an informed consent. No personal information will be disclosed in this study.

Paternity testing
As the Goldeneye™ 20A system exhibited a robustness to a level of forensic biological evidence, the DNA identification was used the Goldey™ 20A kit (People-spot Inc. Beijing, China) following the instructions of the manufacturer. Data analysis such as allelic typing was performed using Gene Marker HID software.

The cumulative parental index (CPI) is defined as:

\[
\text{CPI} = \sum_{i=1}^{n} \text{PI}_i,
\]

Where the paternity index (PI) is: \(\text{PI} = X/Y\), \(\text{PI}_i\) is the paternity index PI when the short tandem repeat (STR) locus is i.

The relative probability of paternity (RPP) = CPI / (CPI + 1) × 100%.

In this case, the detection of 19 autosomal STR loci revealed that the mother and child were in full compliance with Mendel’s law of inheritance at these 19 STR loci, and that the father and child were also in line with Mendel’s law of inheritance at these 19 STR loci. The CPI was 4.5*10⁹ [9], confirming the biological parental relationship.

Discussion
The PHOX2B gene encodes paired-like homeobox 2b protein, which is expressed in the nervous system. Clinically, immunohistochemical staining of PHOX2B protein is a sensitive and specific marker for undifferentiated neuroblastoma [9, 10]. In addition, mutations of PHOX2B gene, both somatic [11] and germline [5], have been reported in previous neuroblastoma studies. In most cases, these mutations are somatic mutations while germline mutations inherited from the patient’s parents are less common. Founder germline mutation of PHOX2B that cause childhood medulloblastoma are even more rare. Here, we identified the c.765_779del deletion of PHOX2B in a patient with medulloblastoma and confirmed that the mutation existed in other tissues from the patient. However, the mutation was absent from his biological parents. Previous studies showed that PHOX2B is associated with neuroblastoma. Meanwhile, our data suggest that the c.765_779del deletion serves as a potential de novo germline mutation that causes medulloblastoma. Further biomolecular studies on PHOX2B are necessary for better understanding its pathogenic role in medulloblastoma.

Recent evidence showed that de novo mutations contribute to a genetic source of cancer causality. Chompert et al. reported that de novo mutations of p53 in childhood cancer are not rare [12]. In addition, a de-novo splice site mutation c.2006-2A > G in the MSH2 gene was found in a young colon cancer patient with negative family history [13]. Paola et al. reported that 38G > A (G13D) is a de novo mutation of NRAS responsible for juvenile myelomonocytic leukaemia [14]. Based on these findings and the purpose of genetic testing in this case (considering having a second child), pathogenic variant screening of parents is informative for making second child decisions. Moreover, in order to aim at better clinical management, it is necessary to well document similar cases and to analyse the difference between novel mutated cases and ordinary cases in terms of pathogenesis, disease development, degrees of clinical severity and prognosis.
| Gene   | Transcript | Nucleotide change | Amino acid change | Mutation frequency | ACMG grade | Patient (Blood) | Patient (Tissue) | Patient (Saliva) | Patient (Urine) | Father | Mother | OMIM/ClinVar |
|--------|------------|-------------------|-------------------|--------------------|------------|----------------|-----------------|----------------|----------------|---------|--------|--------------|
| *MSH2* | NM_000251  | chr2:47637371cA505G | p.I169V          | 0.006361           | likely benign | heterozygous  | heterozygous    | heterozygous    | heterozygous    | no mutation | heterozygous | Lynch syndrome, Turcot syndrome Mismatch repair cancer syndrome [6] |
| *MEI12*| NM_005120  | chrX:70360579cA6139G | p.I2047V         | 0.001032           | uncertain significance | homozygous | homozygous    | homozygous    | low concentration, unable to detect | no mutation | heterozygous | Lujan-Fyns syndrome [7], Ohdo syndrome, Opitz-Kaveggia syndrome |
| *PHOX2B*| NM_003924  | chr4:41747990–41, p.255_260del - 7'48004, c.765_779del, (rs761018157) | p.255_260del - likely benign | heterozygous | heterozygous | heterozygous    | low concentration, unable to detect | no mutation | No mutation | Neuroblastoma [8], Hirschsprung disease |
Table 2 Primers design

| Gene   | Exon   | Forward primer               | Reverse primer               |
|--------|--------|-------------------------------|------------------------------|
| MSH2   | Exon3  | GATAGTCAGCTTCCATTTGTTTG       | GGCCTGGAACTCCTCTCTATCACTA    |
| MED12  | Exon42 | CAGGTCAGGGACCAAGGTTTATAC      | CAATGTCCAAACCTCCTCCACACAT    |
| PHOX2B | Exon3  | CAGATCAGAACATACGTCTCTCCTACT   | GCAAGTTGGCAAGCAGGAGG         |

Conclusion
In this case, we reported a de novo PHOX2B germline mutation as a potential cause of medulloblastoma in a child. Familiar germline variant screening is a recommended tool when an affected family is considering having a second child.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13053-021-00170-5.

Additional file 1.

Abbreviations
WES: Whole exome sequencing; PCR: Polymerase chain reaction; CPI: Cumulative parental index; PI: Paternity index; STR: Short tandem repeat; RPP: Relative probability of paternity

Authors’ contributions
JX Z and HM L conceived and designed the research. XS S, and CP K performed the case analysis and clinical interpretation of genetic test data. CP K, HM L, CM L, BF J, and JX Z managed the patient. A ML C, KL H, and CP K performed the case analysis and clinical interpretation of genetic test data. CP K, HM L, CM L, BF J, and JX Z managed the patient. A ML C, KL H, and JX Z were responsible for the main work of the study and publication is approved by all authors.

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate
The Medical Ethics Committee of the Maoming People’s Hospital reviewed and approved this study. Informed consent was obtained from parents and the patient in the study.

Consent for publication
All authors have read the manuscript and approved for publication.

Competing interests
The authors declare that they have no conflict of interest.

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