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Clinic-pathological description of three paediatric medulloblastoma cases with *MLL2/3* gene mutations

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Medulloblastoma is the most common paediatric malignant tumour. To identify altered genetic events in a comprehensive manner, we recently performed exome sequencing of a series of medulloblastomas [1]. This study identified mutations in genes involved in chromatin modification in 20% of patients examined, including the *myeloid/lymphoid or mixed lineage leukemia (MLL)* family genes *MLL2* and *MLL3*, which were not previously known to be associated with medulloblastoma [1]. The majority of those alterations were nonsense or frameshift mutations, indicating that *MLL2* and *MLL3* are new medulloblastoma tumour suppressor genes [1]. Subsequent exome sequencing studies further validated *MLL2* pathway mutations as medulloblastoma driver events [2-4]. In this report, we present detailed histopathological characteristics of three cases with *MLL2/3* gene mutations.

The male patient discussed in case #1 initially presented as a 5-year-old with a profound frontal headache associated with nausea and vomiting, following receipt of an immunization booster. Five days later the headache returned, and he was noted to have a gait imbalance; a magnetic resonance imaging scan showed a fourth ventricular mass (Figure 1A).

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Histopathological analysis revealed a medulloblastoma. Therapy consisted of craniospinal irradiation with a posterior fossa boost and chemotherapy consisting of a bone marrow transplant protocol of vincristine, amifostine, cisplatin, and cyclophosphamide. He is now 5 years post therapy without evidence of disease.

Case #2 is of a male patient who presented as an 11-year-old who began to experience decreased appetite and headaches that awoke him, associated with nausea and vomiting. A computed tomography scan showed marked hydrocephalus with a 4 cm mass in the posterior fossa. Histopathological analysis identified a medulloblastoma. Post-operatively, he underwent cranio-spinal radiation therapy and chemotherapy with vincristine, cisplatin, and cyclophosphamide supplemented with hyperalimentation via gastric tube placement. Now at six years post-diagnosis, he is doing well at recent follow-up.

Case #3 is a female patient who presented as a 7-year-old with a three-week history of headache associated with morning nausea and vomiting, dizziness and recent onset of double vision. Radiographic studies revealed an enhancing mass lesion in the fourth ventricle. Axial and sagittal gadolinium-enhanced images demonstrated diffuse leptomeningeal spread of disease. Histopathological analysis disclosed a medulloblastoma. Cytological examination of her post-operative cerebrospinal fluid revealed malignant cytology. The patient began craniospinal X-ray therapy. Three months following initial diagnosis, she died of disease. Postmortem examination of the brain and spinal cord revealed extensive spread along the subarachnoid space of the cerebellum, forebrain, brain stem, and spinal cord.

The term medulloblastoma describes a series of heterogeneous brain tumours originating in the cerebellum. This heterogeneity is reflected at two levels: (1) tumours are histopathologically and molecularly distinct; (2) there is a lack of tight correlation between histopathological and molecular subtypes, as tumours within each histopathological subtype are also molecularly heterogeneous. Accordingly, additional genetic alterations, and analysis of the histopathological characteristics associated with them, may provide information for improving tumour subclassification. As a first step toward that purpose, we present three medulloblastoma cases with *MLL2/3* mutations. Intriguingly, all three cases demonstrate features of a moderate to severe large-cell/anaplastic subtype (Figure 1B). However, despite these similarities, clinical outcomes varied. Patient #3 had both *MLL2* and *MLL3* mutations and, unlike the first two patients, had a poor clinical outcome. However, Patient #3 also had *MYC* amplification (frequently associated with a poor prognosis [5]).

The role of *MLL2/MLL3* complexes in medulloblastoma are unknown, yet genetic and biological evidence supports a tumour suppressor role [1-4, 6], and studies have identified *MLL2/3* gene mutations in a variety of other cancers. *MLL* family genes are essential for histone modification and play roles in regulating other developmentally critical pathways [7, 8]. One of these pathways impacted by *MLL2*, retinoic acid signaling [9], may in turn impact *orthodenticle homeobox 2 (OTX2)* expression [10]. Because increased *OTX2* expression was noted (Table 1, Figure 1C), it is tempting to postulate that *MLL2/3* inactivation, and the subsequent changes in histone methylation, may present a mechanism for *OTX2* overexpression, and thus dysregulation of *OTX2*-associated pathways.

Additionally, it is possible that loss of MLL2/MLL3 function impairs cell differentiation and renders cells susceptible to transformation. All cases presented here demonstrated anaplastic features, geographic necrosis and characteristics of the same histopathological subclass. Molecular subclassification, completed for Cases #1 and #2, revealed Group 3 classification for both cases (classification based on Northcott et al. 2011[11]). Because of the presence of *MYC* amplification and the extremely poor prognosis, it is likely that the tumour in Case #3 is also a Group 3 tumor.

It is expected that improved subclassification will provide guidance for therapy and risk assessment in the clinical setting. *MLL2/3* mutations add one more genetic variable for subclassification of medulloblastomas. *MLL2/3* pathway mutations were found to be distributed among various histological groups in previous studies [2, 4]. Additionally, studies have found *MLL2/3* mutations to be distributed among various molecular subgroups [2-4]. To clarify the subclassification issue, more detailed histopathological analysis of a large number of patients with *MLL2/3* mutations will be necessary. We favour the possibility that dysregulation of the *MLL2/3* pathway affects the histopathological and clinical characteristics of medulloblastoma, and we suggest an analysis of more cases is warranted.

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Abbreviations

MLL2	myeloid/lymphoid or mixed lineage leukemia 2
MLL3	myeloid/lymphoid or mixed lineage leukemia 3
2-OTX2	orthodenticle homeobox

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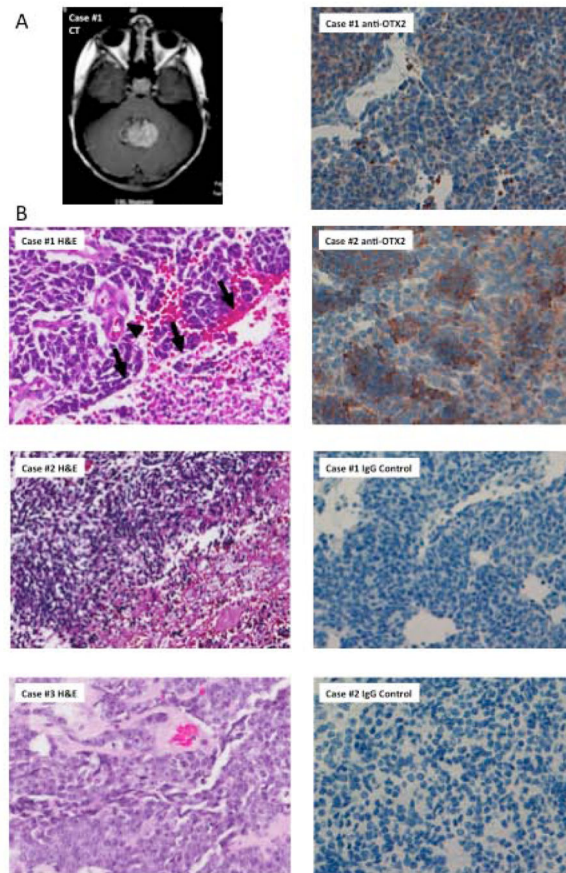


Figure.

A) CT demonstrates a hyperdense 3x4 centimeter midline posterior fossa mass that appears to fill the fourth ventricle, containing punctate calcifications and cystic components. B) Haematoxylin and eosin staining of histological sections from Case #1, Case #2, and Case #3. Note that Case #1 demonstrates hyperchromatic, molded nuclei associated with vascular proliferation (arrowhead) and geographic necrosis (arrows) indicative of a severely anaplastic medulloblastoma; geographic necrosis also suggests a lack of differentiation. C) Immunohistochemical assay shows positive OTX2 expression in Case #1 (Score:40% 2+) and Case #2 (Score: 50% 2+).

Case	Age	Gender	MLL2/3 Mutation*	PTCH/CTNNB1/TP53/PTEN Status	MYC Amplification*	OTX2 Amplification*	OTX2 Expression	Histopathologic Subtype	Survival
1	5	Male	MLL2: missense (R4852Q)	no mutation	No	No	Positive	Severe Anaplastic	5 years, alive
2	11	Male	MLL2: Frameshift	no mutation	ND	ND	Positive	Moderate Anaplastic	6 years, alive
3	7	Female	MLL2: Nonsense (R4921X); MLL3: Nonsense (R2609X)	no mutation	Yes	No	ND	Large Cell	3 months, deceased