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GENETIC ANALYSIS OF TP53 AND CTNNB1 MUTATIONS IN PEDIATRIC MEDULLOBLASTOMA: A STUDY FROM KAZAKHSTAN

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Abstract

Background: Medulloblastoma is the most common malignant pediatric brain tumor, exhibiting significant genetic heterogeneity. Mutations in TP53 and CTNNB1 have been implicated in tumorigenesis, prognosis, and treatment response. However, their precise role in medulloblastoma remains incompletely understood. This study aimed to analyze TP53 and CTNNB1 variants in Kazakhstani pediatric medulloblastoma patients and assess their clinical significance.

Methods: A retrospective analysis was conducted on 33 pediatric medulloblastoma cases from 2015 to 2023. Formalin-fixed paraffin-embedded (FFPE) tumor tissues were used for DNA extraction and Sanger sequencing of TP53 (exons 4, 5, 6, 7, 8) and CTNNB1 (exons 3, 4). Genetic variants were classified using ClinVar, ACMG guidelines, and AMP classification. Statistical associations between mutations, clinical features, and outcomes were assessed.

Results: Among 28 successfully sequenced cases, seven TP53 variants were identified: c.214_215delinsTG (p.Pro72Cys), c.215_216delinsGT (p.Pro72Arg), c.300G>A (p.Gln100=), c.356C>G (p.Ala119Gly), c.357C>G (p.Ala119=), c.782+10C>G (splice-region), and c.817C>G (p.Arg273Gly). The p.Pro72Arg variant was significantly associated with metastasis ($p = 0.008$). The c.356C>G (p.Ala119Gly) variant, previously linked to Li-Fraumeni syndrome, was detected in one case. No variants were found in exons 3 and 4 of CTNNB1.

Conclusion: This study highlights the role of TP53 mutations in medulloblastoma progression, particularly their association with metastasis. The absence of CTNNB1 mutations suggests that WNT pathway activation may be rare in this cohort. Further studies with larger sample sizes and functional validation are needed to clarify the prognostic and therapeutic implications of TP53 mutations in pediatric medulloblastoma.

Keywords: *Medulloblastoma, TP53, CTNNB1, pediatric brain tumor, genetic mutations, Sanger sequencing.*

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Резюме

ГЕНЕТИЧЕСКИЙ АНАЛИЗ МУТАЦИЙ TP53 И CTNNB1 ПРИ МЕДУЛЛОБЛАСТОМЕ У ДЕТЕЙ: ИССЛЕДОВАНИЕ В КАЗАХСТАНЕ

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Введение: Медуллобластома — самая распространённая злокачественная опухоль головного мозга у детей, характеризующаяся значительным генетическим разнообразием. Мутации в генах TP53 и CTNNB1 играют роль в опухолеобразовании, прогнозе и ответе на лечение. Однако их точная роль при медуллобластоме до конца не изучена. Целью настоящего исследования явилось изучение вариантов TP53 и CTNNB1 у казахстанских детей с медуллобластомой и оценка их клинического значения.

Методы: Был проведён ретроспективный анализ 33 случаев детской медуллобластомы за период с 2015 по 2023 год. Для экстракции ДНК и последующего секвенирования по Сэнгеру генов TP53 (экзоны 4, 5, 6, 7, 8) и CTNNB1 (экзоны 3, 4) использовались опухолевые ткани, фиксированные в формалине и залитые в парафин (FFPE). Генетические варианты классифицировались согласно базе данных ClinVar, рекомендациям ACMG и классификации AMP. Статистическая связь между мутациями, клиническими характеристиками и исходами была проанализирована.

Результаты: Из 28 успешно секвенированных образцов были выявлены семь вариантов TP53: c.214_215delinsTG (p.Pro72Cys), c.215_216delinsGT (p.Pro72Arg), c.300G>A (p.Gln100=), c.356C>G (p.Ala119Gly), c.357C>G (p.Ala119=), c.782+10C>G (область сплайсинга) и c.817C>G (p.Arg273Gly). Вариант p.Pro72Arg показал значимую ассоциацию с метастазированием (p = 0.008). Вариант c.356C>G (p.Ala119Gly), ранее связанный с синдромом Ли-Фраумени, был выявлен в одном случае. В экзонах 3 и 4 гена CTNNB1 мутаций не обнаружено.

Заключение: Данное исследование подчёркивает роль мутаций TP53 в прогрессировании медуллобластомы, в особенности их связь с метастазированием. Отсутствие мутаций в CTNNB1 указывает на редкость активации WNT-пути в данной когорте. Для более точной оценки прогностического и терапевтического значения мутаций TP53 при детской медуллобластоме необходимы дальнейшие исследования с большим числом пациентов и функциональной валидацией.

Ключевые слова: *медуллобластома, TP53, CTNNB1, опухоль головного мозга у детей, генетические мутации, секвенирование по Сэнгеру.*

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Түйіндеме

TP53 ЖӘНЕ CTNNB1 МУТАЦИЯЛАРЫНЫҢ БАЛАЛАРДАҒЫ МЕДУЛЛОБЛАСТОМАСЫНДА ГЕНЕТИКАЛЫҚ ТАЛДАУЫ: ҚАЗАҚСТАНДА ЗЕРТТЕУ

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Кіріспе: Медуллобластома — балалар арасында ең жиі кездесетін қатерлі ми ісігі болып табылады және генетикалық тұрғыдан алғанда айтарлықтай әртүрлілікпен сипатталады. TP53 және CTNNB1 гендеріндегі мутациялар ісіктің пайда болуына, болжамына және емге жауап беруіне әсер етуі мүмкін. Алайда, бұл мутациялардың медуллобластома кезіндегі нақты рөлі толық анықталмаған. Осы зерттеудің мақсаты — Қазақстандық балалар арасындағы медуллобластома жағдайларында TP53 және CTNNB1 гендерінің варианттарын талдап, олардың клиникалық маңыздылығын бағалау.

Әдістер: 2015 жылдан 2023 жылға дейінгі аралықта балалар арасындағы 33 медуллобластома жағдайына ретроспективті талдау жүргізілді. Формалинмен бекітіліп, парафинге салынған (FFPE) ісік тіндерінен ДНҚ бөліп

алынып, TP53 (4, 5, 6, 7, 8-экзондар) және CTNNB1 (3, 4-экзондар) гендеріне Сэнгер секвенирлеуі жүргізілді. Генетикалық варианттар ClinVar дерекқоры, ACMG нұсқаулығы және AMP классификациясы бойынша сипатталды. Мутациялар мен клиникалық көрсеткіштер арасындағы статистикалық байланыстар талданды.

Нәтижелер: Сәтті секвенирленген 28 жағдайдың ішінде TP53 генінің келесі жеті варианты анықталды: c.214_215delinsTG (p.Pro72Cys), c.215_216delinsGT (p.Pro72Arg), c.300G>A (p.Gln100=), c.356C>G (p.Ala119Gly), c.357C>G (p.Ala119=), c.782+10C>G (сплайсинг аумағы), және c.817C>G (p.Arg273Gly). p.Pro72Arg варианты метастазбен статистикалық түрде сенімді байланыс көрсетті (p = 0.008). Бұған дейін Ли-Фраумени синдромымен байланыстырылған c.356C>G (p.Ala119Gly) варианты бір науқаста анықталды. CTNNB1 генінің 3 және 4-экзондарында ешқандай мутация табылған жоқ.

Қорытынды: Бұл зерттеу TP53 мутацияларының медуллобластоманың өршуіндегі, әсіресе метастазбен байланысындағы рөлін көрсетеді. CTNNB1 мутацияларының болмауы WNT жолының активациясы бұл когортада сирек кездесетінін білдіреді. TP53 мутацияларының болжамдық және терапиялық мәнін нақтылау үшін үлкенірек үлгі көлемімен және функционалдық зерттеулермен қосымша жұмыстар қажет.

Түйінді сөздер: *медуллобластома, TP53, CTNNB1, балалар ми ісігі, генетикалық мутациялар, Сэнгер секвенирлеуі.*

Дәйексөз үшін:

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Introduction

Medulloblastoma, the most common malignant brain tumor in children, represents a significant challenge in pediatric oncology due to its aggressive nature and the complexity of its treatment. Arising from the cerebellum or posterior fossa, this tumor accounts for approximately 20% of all pediatric brain tumors. Its incidence peaks between ages 3 and 8, highlighting a critical period in child development where the impact of both the disease and its treatment can be profound. Despite advancements in multimodal treatment approaches, including surgery, radiation, and chemotherapy, medulloblastoma remains a leading cause of cancer-related morbidity and mortality in children. Recent strides in molecular biology have begun to unravel the genetic and epigenetic underpinnings of this heterogeneous disease, paving the way for more targeted and less toxic therapeutic strategies [12]

However, even in world medicine, the practical use of such approaches is limited. There are currently 4 major genetic types of medulloblastoma with many subtypes, in this connection, some hypotheses have been put forward about the deescalation of therapy [20].

The genetic landscape of pediatric cancers significantly differs from adult cancers, characterized by fewer somatic mutations and a higher frequency of germline alterations in cancer predisposition genes. Pediatric tumors frequently present unique genetic alterations, including gene fusions, enhancer hijacking, and structural variations such as chromothripsis, adding complexity to their genomic profiles. These complexities underscore the need for molecularly targeted diagnostic and treatment strategies [23].

Genetic research plays a pivotal role in advancing the understanding and treatment of medulloblastoma, particularly in pediatric patients. This malignant brain tumor, which primarily affects children, has been linked to several genetic syndromes, including Li-Fraumeni and Gorlin syndromes. However, the specific predisposition genes contributing to medulloblastoma risk remain incompletely defined.

Building on this foundation, recent studies have underscored the importance of germline predisposition in medulloblastoma, revealing that inherited genetic mutations play a more significant role than previously recognized. The largest systematic analysis of genetic susceptibility in medulloblastoma involved screening 110 known cancer predisposition genes in a large patient cohort. They identified six genes, APC, PTCH1, SUFU, TP53, BRCA2, and PALB2, that exhibited significantly higher mutation rates in medulloblastoma patients compared to a control group of 58,000 healthy individuals [23]

Expanding on these findings, ongoing research is now focusing on the molecular properties of medulloblastoma to develop novel therapeutic strategies and refine existing treatment protocols. By further characterizing the genetic landscape of this pediatric brain tumor, scientists aim to uncover additional inherited mutations and epigenetic modifications that may inform both prognosis and treatment approaches. A deeper understanding of the interplay between germline and somatic genetic alterations is crucial for advancing precision medicine in medulloblastoma, with the ultimate goal of improving patient outcomes [25].

Medulloblastoma exemplifies genetic complexity, with four distinct molecular subgroups, WNT, SHH, Group 3, and Group 4, each defined by specific genomic alterations. The genetic mechanisms of Group 3 and Group 4 tumors remain particularly unclear, highlighting the importance of molecular characterization for diagnosis and therapy [19, 26].

Among key genes frequently implicated in pediatric cancers, TP53 and CTNNB1 play critical roles. TP53, a well-known tumor suppressor gene, is frequently mutated in various pediatric tumors, including subsets of medulloblastoma, particularly within the SHH subgroup. Germline TP53 mutations predispose individuals to Li-Fraumeni syndrome, characterized by increased cancer risk, including medulloblastoma. TP53 mutations in medulloblastoma are often linked with chromothripsis events, significantly contributing to tumor pathogenesis and associated with poor clinical outcomes [22, 26].

CTNNB1 encodes β-catenin, a key component of the canonical WNT signaling pathway. Mutations in CTNNB1 are hallmark genetic events in WNT-activated medulloblastomas.

Somatic mutations in exon 3 of CTNNB1 lead to constitutive activation of WNT signaling, promoting uncontrolled cellular proliferation. Notably, the presence of germline APC mutations, responsible for familial adenomatous polyposis, also strongly correlates with the WNT subgroup, although such mutations represent only a small fraction of overall medulloblastoma cases [22, 26].

Considering the pivotal roles of TP53 and CTNNB1 in pediatric tumor biology, targeted sequencing of these genes is crucial for deeper insights into their contributions to tumorigenesis and for improving patient stratification and tailored therapeutic approaches. This study aims to further elucidate the genetic basis and clinical implications of mutations in these critical tumor-associated genes, expanding our understanding of pediatric medulloblastoma genetics [14].

Materials and methods

Sample Collection

We retrospectively randomly selected 33 pediatric cases of medulloblastoma that were surgically removed at the National Scientific Center of Neurosurgery (Astana, Kazakhstan), between 2015 and 2023. These cases were available for analysis. All available specimens consisted of formalin-fixed paraffin-embedded (FFPE) tumor tissues. Histological diagnosis for all cases was reviewed at the Department of Tumor and Diagnostic Pathology, Nagasaki University (Nagasaki, Japan) according to the diagnostic criteria of the WHO classification of tumors of the central nervous system (2021).

Ethical approval for this study was obtained from the Local Ethics Committee of Astana Medical University (№24 of November 11, 2022 of Protocol №2), and written informed consent was obtained from the parents or legal guardians of all patients in accordance with the Declaration of Helsinki.

DNA Extraction

For the analysis of p53 and CTNNB1 mutations, genomic DNA (gDNA) was individually extracted from FFPE tissues by macrodissection. Each 10 μm thick FFPE section was deparaffinized in 80% xylene in a tube. The deparaffinized sample was then washed twice with absolute ethanol and centrifuged at 15,000 g for 15 minutes at 20°C. After drying, the samples were digested with proteinase K overnight at 56°C. DNA extraction was performed using the Maxwell RSC DNA FFPE Kit (Promega, Madison, Wisconsin, USA) and the Maxwell RSC Instrument (Promega) according to the manufacturer's protocol. The concentration of double-stranded DNA was quantitatively determined using the QuantiFluor ONE dsDNA system (Promega).

Sequencing and bioinformatics

For the analysis of gene mutations, MB components were microdissected from each FFPE section and transferred separately into tubes. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and checked for genetic alterations. Details of the PCR conditions for detecting genetic alterations are summarized in Table 1.

Table 1.

Details of Genetic Alteration Analyses.

Gene	Exon	Primer sequence	Annealing temperature, *C	Amplication size, bp	# of cycles
p53	Exon 4	5'-TCTGACTGCTCTTTTCACCCA-3'	60	360	35
		5'-TGGAAGCCAGCCCCTCAG-3'			
	Exon 5	5'-ACGCCAACTCTCTCTAGCTC-3'	60	540	35
		5'-AACCAGCCCTGTCGTCTC-3'			
	Exon 6	5'-CTGCTGCTTATTTGACCTCCC-3'	60	190	35
		5'-ATCTCCCAACCTCGTGATCC-3'			
	Exon 7	5'-TTGGGCCTGTGTTATCTCCT-3'	60	300	35
		5'-GGGTCAGAGGCAAGCAGA-3'			
	Exon 8	5'-GGACCTGATTTCCTTACTGCC-3'	60	300	35
		5'-GCTTCTTGTCCTGCTTGCTT-3'			
CTNNB 1	Exon 3	5'-TCTGCTTTTCTTGGCTGTCT-3'	63	480	45
		5'-GTTCTCAAAACTGCATTCTGACT-3'			
	Exon 4	5'-TGCTGAACTGTGGATAGTGAGT-3'	63	480	45
		5'-TCCCCTTGAGCATTTACTTCAA-3'			

Amplicon aliquots were treated with Exo-SAP-IT (USB Corp., Cleveland, OH, USA) and sequenced on an ABI Prism 3130 automated capillary DNA sequencer (Applied Biosystems, Foster City, California, USA) using the BigDye Terminator cycle sequencing ready reaction kit 3.1 (Applied Biosystems).

Statistical analyses. Statistical analyses were performed using Jamovi software (version 2.6.17). Categorical variables were analyzed using or Chi-square tests. The association between genetic variants and clinical characteristics, including tumor stage, metastasis, therapy response, and survival outcomes, was assessed. A p-value <0.05 was considered statistically significant

Results

Total 33 pediatric patients diagnosed with medulloblastoma were included in this study (Table 2). The mean age at diagnosis was 8.64 years (SD = 4.25, range = 1–17 years, median = 8 years). The cohort consisted of 22 males (66.7%) and 11 females (33.3%).

Tumor staging was determined according to the TNM classification. Two patients (6.1%) were classified as T1, 15 patients (45.5%) as T2, three patients (9.1%) as T3A, ten patients (30.3%) as T3B, and three patients (9.1%) as T4. Metastatic disease was observed in nine patients (27.3%), with 24 patients (72.7%) classified as M0, one patient (3.0%) as M1, two patients (6.1%) as M2, and six patients (18.2%) as M3.

Following tumor resection, tumor size classification was recorded. Thirteen patients (39.4%) had S1 tumors, ten patients (30.3%) had S2 tumors, eight patients (24.2%) had S3 tumors, and two patients (6.1%) had S4 tumors. Magnetic resonance imaging (MRI) volumetric assessment showed that 14 patients (42.4%) had no residual tumor (0 cm^3), while eight patients (24.2%) had a residual tumor volume of 1 cm^3, nine patients (27.3%) had 2 cm^3, and two patients (6.1%) had 4 cm^3.

Table 2.

The clinical characteristics of pediatric medulloblastoma patients in Kazakhstan.

Variable		n	%
Gender	Male	22	33.3
	Female	11	66.7
Initial tumor size	T1	2	6.1
	T2	15	45.5
	T3A	3	9.1
	T3B	10	30.3
	T4	3	9.1
Metastasis	M0	24	72.7
	M1	1	3.0
	M2	2	6.1
	M3	6	18.2
Size after surgery	S1	13	39.4
	S2	10	30.3
	S3	8	24.2
	S4	2	6.1
Volume by MRI	0	14	42.4
	1	8	24.2
	2	9	27.3
	4	2	6.1
Histology	Classic	10	30.3
	Nodular	21	63.6
	Extensive nodular	2	6.1
Treatment results	Remission	15	46.9
	Stabilization	13	40.6
	Progression	4	12.5
Outcome	Alive	28	84.8
	Dead	5	15.2

Histopathological analysis revealed that ten patients (30.3%) had the classic medulloblastoma variant, while the majority of patients (63.6%) had the nodular variant. Two patients (6.1%) had the extensive nodular variant.

Patients underwent different treatment regimens, including chemotherapy alone, which was administered to seven patients (21.2%), chemotherapy combined with radiotherapy, which was given to 23 patients (69.7%), and chemotherapy with radiotherapy followed by hematopoietic cell transplantation, which was used in three patients (9.1%). At the time of follow-up, 15 patients (46.9%) had achieved remission, while 13 patients (40.6%) exhibited disease stabilization, and four patients (12.5%) experienced disease progression. Overall, 28 patients (84.8%) were alive, while five patients (15.2%) had succumbed to the disease.

DNA sequencing was successfully performed on 28 patients, as five samples were excluded due to poor DNA quality. Analysis of CTNNB1 revealed no any variants in exons 3 and 4. Among the 28 patients whose DNA sequencing was successfully performed, several variants in the TP53 gene were identified. These variants included missense mutations, synonymous changes, and splice-region alterations, primarily located in exons 4, 7, and 8 (Tables 3 and 4). The most frequently detected variants were c.300G>A (p.Gln100=) and c.215_216delinsGT (p.Pro72Arg) in exon 4, with frequencies of 0.64 and 0.68, respectively. Other detected variants included c.214_215delinsTG (p.Pro72Cys), c.356C>G (p.Ala119Gly), c.357C>G (p.Ala119=), c.782+10C>G (splice-region variant in exon 7), and c.817C>G (p.Arg273Gly) in exon 8.

The classification of TP53 variants based on AMP, ACMG, and ClinVar criteria indicated that c.817C>G (p.Arg273Gly) was pathogenic, while c.356C>G (p.Ala119Gly) was likely pathogenic. Other variants, including c.214_215delinsTG, c.215_216delinsGT, and c.357C>G, were categorized as variants of uncertain significance (VUS). The c.300G>A variant was classified as likely benign, with low oncogenic support.

The analysis of TP53 variants in 28 patients revealed significant differences in the distribution of mutations across clinical and pathological variables (Table 5). Among the four most frequently identified variants, c.214_215delinsTG, c.215_216delinsGT, c.300G>A, and c.357C>G, there were no statistically significant differences in their occurrence between male and female patients. The presence of these variants also did not show a significant correlation with patient age, as the distribution remained relatively uniform across different age groups.

Table 3.

Identified variants in TP53 gene (N=28).

Genetic variant	Protein sequence variant	rs	Variant type	Location	f (study)	f (population)
c.214_215delinsTG	p.Pro72Cys	rs730882014	Missense	Exon 4	0.25	N/A
c.215_216delinsGT	p.Pro72Arg	rs878854066	Missense	Exon 4	0.68	N/A
c.300G>A	p.Gln100=	rs1597373952	Synonymous	Exon 4	0.64	N/A
c.356C>G	p.Ala119Gly	rs2073451331	Missense	Exon 4	0.036	N/A
c.357C>G	p.Ala119=	-	Synonymous	Exon 4	0.18	N/A
c.782+10C>G	-	-	Splice region	Exon 7	0.036	N/A
c.817C>G	p.Arg273Gly	rs121913343	Missense	Exon 8	0.071	N/A

Metastasis status was significantly associated with the presence of TP53 variants. Patients with metastatic disease (M1–M3) had a significantly higher frequency of the c.214_215delinsTG variant (66.7%) compared to non-metastatic patients (M0) (13.6%; $p = 0.008$). Similarly, the c.215_216delinsGT and c.300G>A variants were more frequently observed in patients with metastasis, with statistically significant associations ($p = 0.041$ and $p = 0.006$, respectively). However, no significant correlation was observed between metastasis and the c.357C>G variant ($p = 0.264$).

Table 4.

Classification of identified variants in TP53 gene.

Genetic variant	AMP classification	Oncogenic classification	ACMG classification	ClinVar Clinical Significance
c.214_215delinsTG	Tier 2	Uncertain	VUS	Uncertain
c.215_216delinsGT	Tier 2	Moderate Oncogenic Support	VUS	Likely benign
c.300G>A	Tier 3	Low Oncogenic Support	Likely benign	Likely benign
c.356C>G	Tier 1	Low Oncogenic Support	Likely Pathogenic	Likely Pathogenic-VUS
c.357C>G	Tier 2	Uncertain	VUS	-
c.782+10C>G	Tier 3	Uncertain	VUS	-
c.817C>G	Tier 1	Oncogenic	Pathogenic	Pathogenic

Table 5.

Distribution of TP53 variants across clinical and pathological variables.

Variable	c.214_215delinsTG		c.215_216delinsGT		c.300G>A		c.357C>G	
	n (%)	χ^2, p	n (%)	χ^2, p	n (%)	χ^2, p	n (%)	χ^2, p
Gender		0.207,				0.221,		0.05,
Male (n=18)	5 (27.8%)	p=0.649	12 (66.7%)		11 (61.1%)	p=0.638	3 (16.7%)	p=0.825
Female (n=10)	2 (20.0)		7 (70.0%)		7 (70.0%)		2 (20.0%)	
Age group		-		0.109,		0.233,		1.01,
< 4 years (n=4)	1 (25.0%)		3 (75.0%)	p=0.741	3 (75.0%)	p=0.629	0	p=0.314
4-18 years (n=24)	6 (25.0%)		16 (66.7%)		15 (62.5%)		5 (17.9%)	
Metastasis		7.07,		4.17,		7.54,		1.25,
M0 (n=22)	3 (13.6%)	p=0.008	17 (77.3%)	p=0.041	17 (77.3%)	p=0.006	3 (13.6%)	p=0.264
M1-3 (n=6)	4 (66.7%)		2 (33.3%)		1 (16.7%)		2 (33.3%)	
Histology		3.86,		4.93,		7.71,		0.551,
Classic (n=8)	4 (50.0%)	p=0.145	3 (37.5%)	p=0.085	2 (25.0%)	p=0.021	2 (25.0%)	p=0.759
Nodular (n=19)	3 (15.8%)		15 (78.9%)		15 (78.9%)		3 (15.8%)	
Extensive Nodular (n=1)	0		1 (100%)		1 (100%)		0	
Therapy		0.351,		2..30,		2.21,		0.790,
CTX (n=6)	1 (16.7%)	p=0.839	5 (83.3%)	p=0.316	5 (83.3%)	p=0.331	1 (16.7%)	p=0.674
CTX + RTX (n=19)	5 (26.3%)		13 (68.4%)		12 (63.2%)		4 (21.1%)	
CTX + RTX + HCT (n=3)	1 (33.3%)		1 (33.3%)		1 (33.3%)		-	
Treatment results		1.40,		1.88,		1.99,		1.04,
Remission (n=12)	2 (25.0%)	p=0.497	7 (58.3%)	p=0.392	7 (58.3%)	p=0.371	3 (25.0%)	p=0.594
Stabilization (n=12)	4 (33.3%)		8 (66.7%)		7 (58.3)		2 (16.7%)	
Progression (n=3)	0		3 (100%)		3 (100%)		0	
Outcome		1.56,		2.21,		2.59,		1.01,
Alive (n=24)	7 (29.2%)	p=0.212	15 (62.5%)	p=0.137	14 (58.3%)	p=0.107	5 (20.8%)	p=0.314
Dead (n=4)	0		4 (100%)		4 (100%)		0	

Histological subtype analysis showed that the c.214_215delinsTG variant was more frequently observed in patients with the classic subtype (50.0%) compared to the nodular subtype (15.8%), although this association did not reach statistical significance (p = 0.145). A similar trend was observed for the c.215_216delinsGT and c.300G>A variants, with higher frequencies in the nodular subtype, but only the latter variant showed a statistically significant association (p = 0.021). The c.357C>G variant did not demonstrate a significant association with histological subtypes (p = 0.759).

Evaluation of treatment regimens indicated no statistically significant associations between TP53 variants and the type of therapy received. Patients treated with chemotherapy alone, chemotherapy with radiotherapy, and chemotherapy combined with hematopoietic cell transplantation exhibited similar frequencies of TP53 variants. Similarly, treatment response outcomes, including remission, disease stabilization, and progression, were not significantly associated with the presence of the analyzed TP53 variants.

Survival analysis showed that patients harboring the c.215_216delinsGT and c.300G>A variants had a higher prevalence among those who died compared to survivors. However, the observed differences did not reach statistical significance (p = 0.137 and p = 0.107, respectively). The c.214_215delinsTG and c.357C>G variants were found exclusively in surviving patients, but without statistical significance.

Further individual patient analysis revealed that one patient carrying the c.356C>G variant was a male over four years old with a T2, M0 tumor of the classic histological subtype, who achieved remission following chemotherapy, radiotherapy, and hematopoietic cell transplantation and remained alive at follow-up. Another patient harboring the c.782+10C>G splice-region variant was a male over four years old with a T1, M0 nodular tumor, who also achieved remission following chemotherapy and radiotherapy. Two patients with the c.817C>G variant were female, over four years old, and had T2, M0 tumors with either a classic or

nodular histological subtype, both of whom achieved remission following chemotherapy and radiotherapy

Discussion

Medulloblastoma is the most common malignant pediatric brain tumor, with a highly heterogeneous molecular and clinical profile. In this study, we analyzed 33 pediatric patients with medulloblastoma to assess their clinical characteristics, treatment outcomes, and the presence of TP53 and CTNNB1 gene variants. The median age at diagnosis was 8 years, with a male predominance (66.7%). Tumor classification revealed that the majority of cases were T2 (45.5%) or T3B (30.3%), with metastatic disease present in 27.3% of patients. Treatment regimens varied, with most patients receiving a combination of chemotherapy and radiotherapy (69.7%), while hematopoietic cell transplantation was used in a small subset (9.1%). At the time of follow-up, 46.9% of patients had achieved remission, while 15.2% had succumbed to the disease. Genetic analysis identified several TP53 mutations, including missense and splice-region variants primarily located in exons 4, 7, and 8, with significant associations observed between specific mutations and metastasis. Importantly, no any variants were identified in exons 3 and 4 of CTNNB1, suggesting that mutations in these regions do not play a major role in this cohort. These findings contribute to the growing understanding of the molecular landscape of medulloblastoma and its potential impact on disease progression and patient outcomes.

The Role of TP53 in Medulloblastoma

TP53 is one of the key tumor-suppressor genes encoding the p53 protein, known as the "guardian of the genome." It plays a crucial role in regulating the cell cycle, apoptosis, DNA repair, and other processes that protect cells from carcinogenic mutations. Mutations in TP53 disrupt its function, leading to uncontrolled cell growth [8]

Familial Forms: TP53 mutations can be associated with hereditary syndromes, such as Li-Fraumeni syndrome. Children with this syndrome have an increased risk of developing various types of cancer, including medulloblastoma. This is due to the impaired functionality of p53, making brain cells more susceptible to transformation [11].

Prognosis and Clinical Course: TP53 mutations are linked to a more aggressive course of medulloblastoma. These tumors are often characterized by high levels of aneuploidy (abnormal chromosome numbers), poor prognosis, and a higher recurrence rate. Children with TP53-mutated medulloblastomas may have an increased risk of metastasis and a suboptimal response to standard therapies [24].

Prognostic Markers: In some cases, the presence of TP53 mutations can serve as an indicator of a high risk of tumor recurrence or metastasis, which is crucial for determining the intensity of treatment [10].

The Role of β-Catenin in Medulloblastoma

β-Catenin is a key component of the signaling pathway that regulates cell growth and development through the Wnt/β-catenin pathway. Under normal conditions, β-catenin is stabilized in the cytoplasm and activates the transcription of specific genes involved in cell proliferation and differentiation. However, mutations in the CTNNB1 gene can lead to its accumulation in the cell and the activation of oncogenesis-related genes [16].

Mutations and the WNT Subtype: Mutations in the CTNNB1 gene play a crucial role in one of the molecular subtypes of medulloblastoma—the WNT subtype. This subtype is characterized by Wnt/β-catenin pathway activation, promoting cell proliferation and inhibiting apoptosis. CTNNB1 mutations result in persistent β-catenin activation, which can drive tumor growth [9].

Clinical Significance of the WNT Subtype: Medulloblastomas associated with β-catenin mutations generally have a more favorable prognosis compared to other subtypes. These tumors often exhibit a low rate of metastasis and higher long-term survival rates following treatment. Children with this subtype tend to respond well to standard therapies, including surgery, radiation therapy, and chemotherapy [18].

Risk of Recurrence: Although the WNT subtype has a good prognosis, CTNNB1 mutations may still be associated with recurrence in some cases, particularly when treatment does not include targeted therapy aimed at inhibiting Wnt/β-catenin pathway activity [17].

Many ongoing clinical trials have begun to incorporate insights gained from our deeper understanding of the molecular foundations of medulloblastoma: De-escalation of Treatment for WNT Medulloblastoma, Incorporation of Targeted Therapy for SHH Medulloblastoma, Patient Stratification by Subgroup and Disease Risk [13].

Medulloblastoma is a highly heterogeneous pediatric brain tumor with diverse genetic alterations that influence tumor progression, treatment response, and patient outcomes. In this study, we identified several variants in the TP53 gene, including c.214_215delinsTG (p.Pro72Cys), c.215_216delinsGT (p.Pro72Arg), c.300G>A (p.Gln100=), c.356C>G (p.Ala119Gly), c.357C>G (p.Ala119=), c.782+10C>G (splice-region variant), and c.817C>G (p.Arg273Gly). Our findings align with previous studies emphasizing the role of TP53 mutations in pediatric brain tumors and highlight the clinical and functional variability associated with different TP53 variants.

The Pro72Arg (c.215_216delinsGT) variant has been widely studied in different cancer types. This polymorphism has been reported as one of the most common variants in colorectal cancer among Saudi Arabian patients and has been extensively evaluated in the context of cancer risk [1]. However, previous studies have yielded conflicting results regarding its predictive value. *Asadi et al.* (2017) found no significant association between p.Pro72Arg and colorectal cancer risk in the Iranian Azeri population, whereas *Ounalli et al.* (2023) reported that the Pro72 variant was associated with increased susceptibility to chronic lymphocytic leukemia (CLL) and correlated with adverse prognostic markers such as advanced Binet stage, low hemoglobin levels, and thrombocytopenia [2,21]. Moreover, *Bukovac et al.* (2019) reported high prevalence (44.8%) of Pro72 variants among patients with meningiomas [4]. In Li-Fraumeni syndrome, p.Pro72Arg has been associated with earlier tumor onset, with carriers of the Arg allele developing tumors at a younger age than Pro/Pro patients [3]. Although the precise functional consequences of p.Pro72Arg in medulloblastoma remain unclear, its potential impact on tumor progression warrants further investigation.

The c.356C>G (p.Ala119Gly) variant, classified as likely pathogenic, has been detected in individuals with Li-Fraumeni syndrome [5]. While some studies indicate that p.Ala119Gly disrupts TP53 function, experimental data suggest that it remains proficient in growth suppression [6]. This variant is

located in the DNA-binding domain of TP53, a critical region for tumor suppressor activity, and in silico predictions suggest that it may impact RNA splicing. Given that TP53 is frequently mutated in aggressive cancers, further studies are needed to determine whether p.Ala119Gly contributes to tumorigenesis or affects medulloblastoma progression.

The c.817C>G (p.Arg273Gly) variant, located at a mutation hotspot in TP53, has been implicated in various cancers. Li et al. (2014) [15] demonstrated that different p.R273 mutations exhibit distinct functional consequences, with p.R273H and p.R273C promoting tumor cell proliferation, invasion, and drug resistance, whereas p.R273G appears to be less oncogenic. Molecular dynamics simulations suggest that p.R273G has stronger DNA-binding affinity than the other R273 mutations, which may explain its relatively lower oncogenic potential. However, the clinical significance of p.R273G in medulloblastoma remains unclear and requires further validation.

The c.782+10C>G variant is a splice-region alteration, but computational predictions and clinical evidence suggest that it is likely benign [7]. This variant has not been reported in individuals with Li-Fraumeni syndrome, and functional studies have not demonstrated a significant impact on TP53 function. However, given its proximity to a canonical splice site, further functional analyses are necessary to rule out any potential effects on mRNA splicing.

In our study, the presence of TP53 mutations was significantly associated with metastasis, with certain variants, including p.Pro72Cys, p.Pro72Arg, and p.Gln100=, being more frequently observed in metastatic cases. This finding suggests that specific TP53 variants may contribute to tumor dissemination and could serve as potential biomarkers for disease progression. However, no significant associations were found between TP53 mutations and treatment response, highlighting the complexity of TP53's role in medulloblastoma.

One of the limitations of our study is the small sample size, which may have affected the statistical power of our findings. Additionally, the use of FFPE-derived DNA poses challenges in sequencing accuracy due to potential DNA degradation. Further studies with larger cohorts and functional analyses are needed to confirm the impact of these TP53 variants on medulloblastoma prognosis and treatment response.

Conclusion. Our study identified multiple TP53 variants in pediatric medulloblastoma patients, some of which have been previously implicated in cancer progression. The absence of CTNNB1 mutations in exons 3 and 4 suggests that this gene may not play a major role in medulloblastoma pathogenesis in our cohort. Future research should focus on validating the functional consequences of these TP53 variants and exploring their potential as prognostic markers or therapeutic targets in medulloblastoma

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