Original Article

Genomic landscape of medulloblastoma subtypes in an Asian cohort

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Background: Medulloblastoma (MB) is a highly malignant childhood brain tumor. Previous research on the genetic underpinnings of MB subtypes has predominantly focused on European and American cohorts. Given the notable genetic differences between Asian and other populations, a subtype-specific study on an Asian cohort is essential to provide comprehensive insights into MB within this demographic. The aim of this study is to investigate the genomic landscape of MB subtypes in an Asian cohort to better understand the genetic variations and potential implications for clinical practice.

Methods: We conducted a study on an Asian cohort comprising 113 MB patients. Genomic sequencing was performed using MGISEQ-2000 platform. We analyzed the participants' characteristics and compared them with previous studies. All germline variants of the ten susceptibility genes of interest (*APC*, *BRCA2*, *PTCH1*, *PTCH2*, *ELP1*, *SUFU*, *CTNNB1*, *SMARCA4*, *GPR161*, and *TP53*) were annotated and validated.

Results: Our study identified 14 valid germline variants that met our criteria, with these variants being detected in the genes *APC*, *BRCA2*, *PTCH1*, *PTCH2*, *ELP1*, and *SUFU*. Of these, six variants were classified as pathogenic in ClinVar: two in *PTCH2* (c.C1573T), one in *ELP1* (c.C583T), and three in *PTCH1* (c.G1370T, c.C2066T, c.C529T). The remaining eight variants were of uncertain significance, including those in *SUFU* (c.T833C), *ELP1* (c.T2A), *BRCA2* (c.G7488C), and *APC* (c.C3247A, c.A1G, c.A8042G, c.A3056G, c.G822C). Our findings highlight a subtype-based germline variant landscape specific to the Asian cohort and reinforce the connection between *SUFU*, *PTCH1*, and the SHH subtype of MB. Additionally, the identification of ELP1-related cases supports the newest findings in this area and provides typical copy number variation (CNV) results for future investigation.

Conclusions: This study provides valuable insights into the genetic landscape of MB in an Asian cohort, emphasizing the importance of population-specific research. The subtype-specific germline variant landscape identified in this study contributes to the understanding of MB and its genetic underpinnings in Asian populations, potentially guiding future research and therapeutic strategies.

Keywords: Medulloblastoma (MB); genomic landscape; Asian cohort; germline variants; subtype analysis

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Introduction

Medulloblastoma (MB), first proposed by Bailey and Cushing in 1925, is an embryonic tumor that can occur anywhere in the brain, but most often grows in the cerebellar vermis above the roof of the fourth ventricle (1). The World Health Organization (WHO) classifies it as WHO grade IV in the classification of central nervous system (CNS) tumors and pain, indicating that it is histologically highly malignant. Its origin may be related to the non-continued differentiation of various discrete neural stem cells or neural

progenitor cell populations early in life (2).

Epidemiological data suggest that the annual incidence of MB worldwide ranges from 0.20 to 0.58 per one hundred thousand population (3). MB can occur at any age, but the peak age of incidence is 6–8 years old (2), and the overall incidence rate in men is usually 1.5–2 times that of women (4).

In 2007, the World Health Organization (WHO) classified MB into four histological subtypes: classic, desmoplastic nodular (DN), medulloblastoma with extensive nodularity (MBEN), and large cell/anaplastic (LC/A) (5). While these histological subtypes provide some prognostic value, the current gold standard for classification and prognosis is based on molecular subtypes (2,6). According to the 2021 WHO classification (7) of CNS tumors, MB is divided into four primary molecular subtypes: Wingless (WNT), Sonic Hedgehog (SHH), Group 3 (G3), and Group 4 (G4). The WNT and SHH subtypes are defined by specific genetic mutations, such as *CTNNB1* (WNT) and *PTCH*, *SUFU*, *SMO* (SHH). G3 is often associated with high-risk factors like *MYC* amplification, while G4 frequently features i17q alterations (8). Notably, in the 2021 WHO classification, G3 and G4 are increasingly considered a combined non-WNT/non-SHH group due to overlapping molecular characteristics, reflecting their close biological relationship (9).

While most of the current research on the genetic underpinnings of MB subtypes focuses on somatic variants (10), our study targets germline variants. In the field of research on the genetic basis of germline variants in MB subtypes, a pivotal study in 2017 involving a cohort of 102 pediatric patients in Poland highlighted the link between germline variants and MB subtypes, they identified six new potentially pathogenic germline variants in DNA repair genes, including *MSH2*, *RAD50*, and others, which were associated with a significantly higher risk of life-threatening toxicity during chemotherapy in pediatric MB patients (11). However, the connection between typical MB biomarkers (such as *APC*, *PTCH1*, *TP53*, *SUFU*, and *BRCA2*) and molecular subtypes, especially G3 and G4, requires further elucidation. In addition to molecular subtypes, certain genetic syndromes are known to predispose individuals to MB. Gorlin syndrome (also known as basal cell nevus syndrome) is one such syndrome, primarily associated with mutations in the *PTCH1* gene. Other syndromes, such as Turcot syndrome and Li-Fraumeni syndrome, are also known to be linked to MB. Patients with these syndromes typically have a higher risk of developing MB at a young age (12).

The most frequently altered genes are well-known oncogenes and tumor suppressors (e.g., *MYC*, *TP53*, and *PTCH1*) as well as novel MB candidate genes (e.g., *MML2*). In 2015, a risk analysis study involving 243 childhood MB patients and 247 control subjects from Sweden and Denmark was conducted. This study focused on 10 genes that were frequently altered in MB (*CCND2*, *CTNNB1*, *DDX3X*, *GLI2*, *SMARCA4*, *MYC*, *MYCN*, *PTCH1*, *TP53*, and *MLL2/KMT2D*), and the results showed that 8 genetic germline variants were associated with MB risk (13). However, the associations were not statistically significant following Bonferroni correction for multiple comparisons, which may be because this study was overly conservative, as many of germline variants are actually in linkage disequilibrium with each other. In our study, we focused on the following 10 susceptibility genes: *APC*, *BRCA2*, *CTNNB1*, *PTCH1*, *PTCH2*, *SMARCA4*, *GPR161*, *TP53*, *ELP1*, and *SUFU*.

Currently, much of the existing literature is based on European and American cohorts (11,14,15). However, there is a notable gap in molecular subtype-specific research for MB within Asian populations. Consequently, one of our primary objectives is to conduct a subtype-specific study on an Asian cohort, contributing valuable insights to the

Highlight box

Key findings

- This study identifies 14 distinct germline variants in medulloblastoma (MB) patients from an Asian cohort, including six pathogenic variants and eight of uncertain significance, providing the first detailed subtype-based germline variant landscape specific to this population.

What is known and what is new?

- Existing studies on the genomic landscape of MB subtypes have predominantly focused on European and American populations, revealing the presence of common pathogenic germline variants.
- This study highlights a unique germline variant profile in an Asian cohort, with particular emphasis on variants in genes such as *PTCH1*, *SUFU*, and *ELP1*, which are linked to specific MB subtypes, providing insights into population-specific genetic patterns.

What is the implication, and what should change now?

- The findings underscore the importance of considering population-specific genomic data when devising diagnostic and therapeutic strategies for MB. Future research should focus on the functional validation of identified variants and exploring their impact on clinical outcomes to support precision medicine approaches for Asian populations.

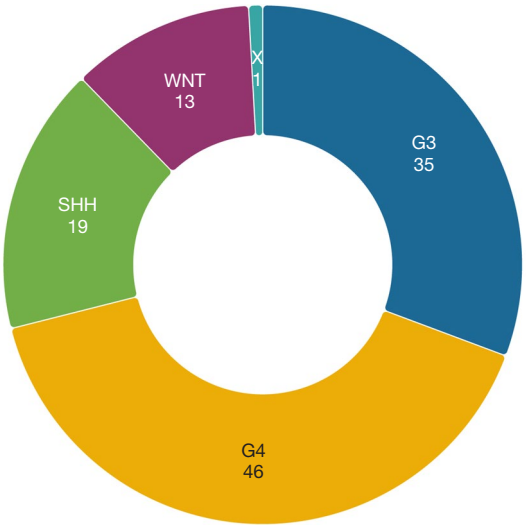

Figure 1 Molecular subtype composition of our cohort (n=114). One sample labeled as 'x' represents an undetermined subtype. WNT, Wingless (medulloblastoma subtype); SHH, Sonic Hedgehog (medulloblastoma subtype); G3, Group 3 (medulloblastoma subtype); G4, Group 4 (medulloblastoma subtype).

understanding of MB in this demographic.

Methods

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The Institutional Review Board of BGI (BGI-IRB 21161-T1). Informed consent was taken from all individual participants or their parents or legal guardians.

Classification of subtype

All subtypes were identified utilizing the Illumina Infinium Methylation EPIC BeadChip arrays, strictly adhering to the protocols provided by the manufacturer. The data were generated from tissue samples, which were either freshly frozen or preserved in formalin-fixed and paraffin-embedded (FFPE) form. Classification of MB subgroups was facilitated by an online platform dedicated to the categorization of CNS tumors based on DNA methylation profiles. This platform is accessible at www.molecularneuropathology.org (version 11b435). The identified subtypes, including WNT, SHH, G3, and G4, were then subjected to further downstream analysis. The identified subtypes in 114 patients (*Figure 1*), including 13 WNT, 19 SHH, 35 G3, and 46 G4, with the subtype of the remaining 1 patient unconfirmed. Then these subtype classifications were used for further downstream analysis (*Figure 2*).

Data generation from whole genome sequencing

Five milliliters of peripheral blood were collected from each participant, and genomic DNA was subsequently extracted utilizing the Qiagen Genomic DNA Extraction Kit (Qiagen, Venlo, The Netherlands). Whole-genome sequencing (WGS) libraries were meticulously constructed for each sample, followed by sequencing on the MGISEQ-2000 platform (DNBSEQ-G400) (16). The size of the reads generated during paired-end sequencing was 150 bp, and the average depth was 30×, while the median depth was 22×.

Annotation of germline variants

Sequencing reads were aligned to the University of California, Santa Cruz (UCSC) human reference genome (hg38) utilizing the Burrows-Wheeler Aligner (BWA), version 0.7.10 (BWA-MEM). Variant identification was performed using two databases, including the Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Functional annotations of the identified germline variants were conducted using AnnoVar (https://annovar.openbioinformatics.org/en/latest/) (17).

Germline variants validation

In our study, germline variants were selected based on specific criteria: they must exhibit at least two deleterious classifications in the Sorting Intolerant From Tolerant (SIFT, https://sift.bii.a-star.edu.sg/), Likelihood Ratio Test (LRT, https://evomics.org/resources/likelihood-ratio-test/) , Mutation Taster (https://www.mutationtaster.org/), or Functional Analysis through Hidden Markov Models (FATHMM) databases (http://fathmm.biocompute.org.uk/), or be classified as Likely Pathogenic or Pathogenic by interVar (https://github.com/WGLab/intervar) according to American College of Medical Genetics (ACMG) guidelines (18). Conversely, variants were excluded if they were labeled as Likely Benign or Benign in ClinVar or interVar, had

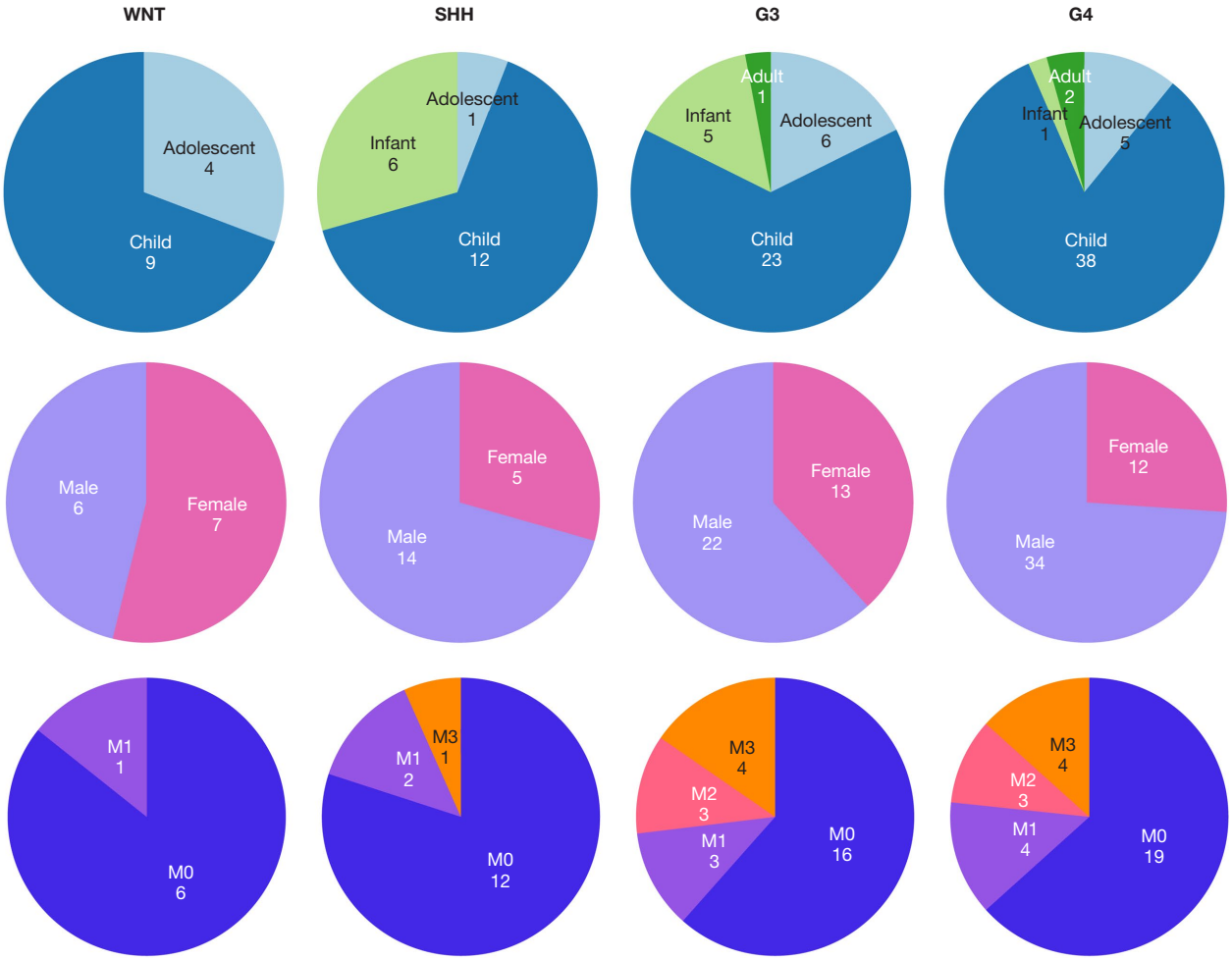

Figure 2 Age group, sex, and metastasis composition of the cohort. Age groups: infant ≤3 years, 3< child <12 years, 12≤ adolescent ≤18 years, adult >18 years. Metastasis status: M0, no metastasis; M+, presence of metastasis.

conflicting interpretations in ClinVar but included cases reported as benign or likely benign, were single nucleotide variants (SNVs) not recorded in either dbSNP or ClinVar, or had a minor allele frequency (MAF) greater than 0.01. After applying the filtering to all variants of ten genes (*APC*, *BRCA2*, *CTNNB1*, *PTCH1*, *PTCH2*, *SMARCA4*, *GPR161*, *TP53*, *ELP1*, and *SUFU*) among 113 patients, 14 valid germline variants passed the criteria.

Copy number variants detection

CNV analysis was performed to explore the relationship between gene variants and their corresponding clinical phenotypes, such as metastasis and recurrence. Identifying CNVs in the regions surrounding variant genes allows for a more comprehensive understanding of their potential functional impacts in tumor development and progression (19). CNVkit, GATK-gCNV, Control-FREEC and CNVpytor are used to analyze CNV result based on our WGS result. Results from four softwares are generated separately and combined to detect more CNV in our result. For further downstream clinical analysis, the result is compared to the result of sample NA12878 in hg38 genome to verify the accuracy.

Statistical analysis

The statistical analysis was performed using R software (version 4.2.0). Descriptive statistics were used to summarize demographic and clinical characteristics of the

Table 1 Summary of basic characteristic of our cohort

Subtype	Male/female	Population	Age (years)	Death rate	M+:M0
SHH	14/5	19	6.06±3.55	3/19	3:12
WNT	6/7	13	9.53±2.72	0/13	1:6
G3	22/13	35	7.93±4.90	7/35	10:16
G4	34/12	46	7.82±3.78	2/46	11:19

Data are presented as n or mean ± SD. SHH, Sonic Hedgehog; WNT, Wingless; G3, Group 3; G4, Group 4; M+, presence of metastasis; M0, absence of metastasis; SD, standard deviation.

patients. Comparisons between different subtypes were conducted using chi-square tests for categorical variables and *t*-tests for continuous variables. Statistical significance was defined as a P value of less than 0.05.

Results

Characteristics of participants

Our cohort comprises 113 MB patients, with their clinical data presented in *Table 1*. Predominantly, our data encompasses patients from G3 and G4. It's important to note that patients lacking information such as metastasis status (M+) or sex were excluded from the analysis but are still part of the overall population.

Our findings align partially with previous studies. Both G3 and G4 exhibit a male-dominant trend, with male-to-female ratios of 1.69 and 2.83, respectively. The WNT subtype presents a nearly equal sex ratio, consistent with previous research. Contrarily, the SHH subtype reveals a surprising male bias, diverging from the expected equal distribution reported in earlier study (9).

In terms of prognosis, our results mirror common trends (*Table 1*). Patients with the WNT subtype demonstrate the most favorable prognosis, characterized by the highest survival rate and the lowest metastasis rate. As anticipated, the G3 group exhibits the lowest survival rate and the highest frequency of metastasis (M+), with the latter slightly surpassing that of the G4 group.

Germline variants clinical interpretation

In the cohort of 113 patients (*Table 1*), comprehensive sequencing of peripheral blood identified 14 distinct germline variants across 14 different patients (*Figure 3*). Of these, six variants were classified as pathogenic according to ClinVar: two in *PTCH2* (c.C1573T), one in *ELP1* (c.C583T), and three in *PTCH1* (c.G1370T, c.C2066T, c.C529T). The remaining eight variants were of uncertain significance, including those found in *SUFU* (c.T833C), *ELP1* (c.T2A), *BRCA2* (c.G7488C), and *APC* (c.C3247A, c.A1G, c.A8042G, c.A3056G, c.G822C). Among these, five variants *APC* (c.A3056G, c.A1G, c.C3247A), *ELP1* (c.T2A), and *SUFU* (c.T833C) were predicted as pathogenic by at least three of the four prediction tools (SIFT, LRT, MutationTaster, FATHMM). Two variants, *APC* (c.A8042G) and *BRCA2* (c.G7488C), were predicted as likely pathogenic by two prediction tools. One variant, *APC* (c.G822C), was predicted as pathogenic by only one tool. Detailed descriptions of these germline variants can be found in *Table 2*.

For the 6 loci recorded as pathogenic in the ClinVar database, intriguingly, three pathogenic germline variants are associated with the *PTCH1* gene and exclusively belong to the SHH subtype. These include two nonsense germline variants located at *PTCH1* c.C529T (p.Q177X) and *PTCH1* c.C2066T (p.A689V). Additionally, a missense germline variant was identified at *PTCH1* c.G1370T (p.G457V). The other two pathogenic germline variants are two nonsense *PTCH2* germline variants, found in the G4 subtype, both involving *PTCH2* c.C1573T (p.R525X).

In addition, we identified two *ELP1* germline variants belonging to the SHH subtype, including a startloss variant at *ELP1* c.T2A (p.M1K) and a nonsense mutation at *ELP1* c.C583T (p.Q195X). Both of these variants are classified as pathogenic in the ClinVar database.

For the variants of uncertain significance (VUS), in the G3 and G4 subtypes, five germline variants of *APC* were identified, all classified as of uncertain significance. These variants are located at *APC* c.A1G (p.M1V), *APC* c.C3247A (p.P1083T), *APC* c.A3056G (p.N1019S), *APC* c.G822C (p.L274F), and *APC* c.A8042G (p.D2681G). Additionally, a *SUFU* variant, classified as of uncertain significance, was identified in the SHH group at *SUFU* c.T833C (p.L278P).

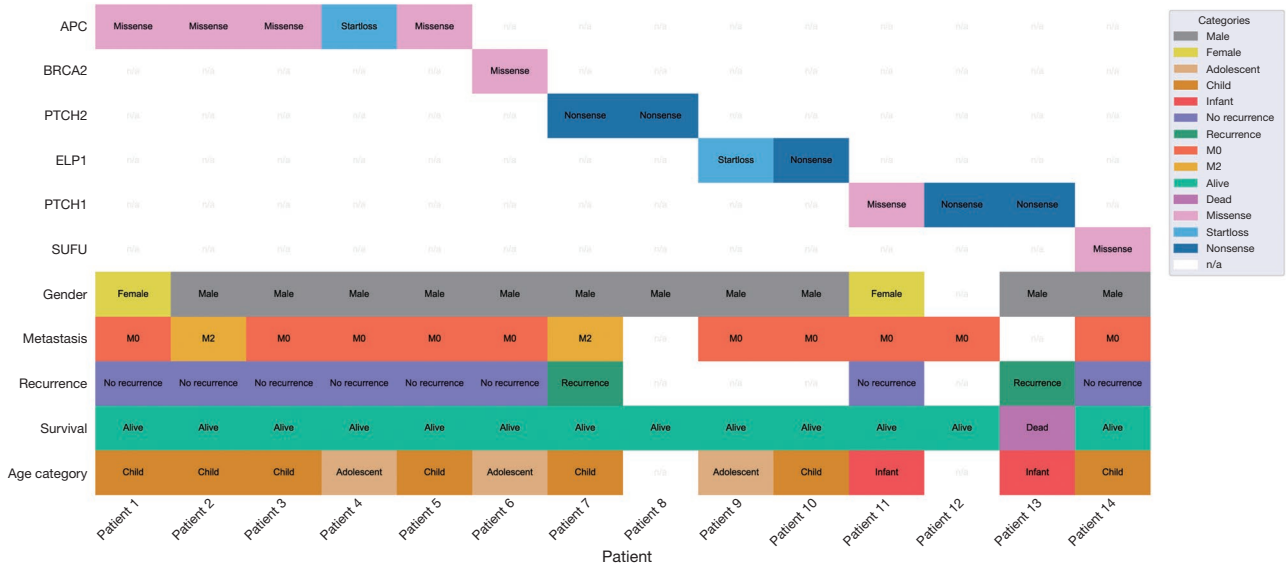

Figure 3 Variant and clinical condition of 14 germline variants identified. Germline variants: identified mutations in specific genes; gender: male or female; metastasis status: M0: no metastasis; M1/M2/M3: different levels of metastasis severity; recurrence: yes or no indicating whether the patient experienced tumor recurrence; age category: infant, child, adolescent, or adult. n/a, not available.

Furthermore, a *BRCA2* germline variant was found in the G4 subtype at *BRCA2* c.G7488C (p.K2496N).

Subtype-related spectrum of germline variants

We analyzed the family history of 14 patients. Among them, Patient 1's grandfather was diagnosed with lung cancer in his fifties. Patient 8's family has a history of various cancers, including skin cancer, gastric cancer, pituitary tumor, colorectal cancer, and cervical cancer, with multiple relatives affected. Additionally, Patient 12 was diagnosed with Gorlin syndrome and basal cell carcinoma. Patient 14's grandfather had lung cancer. Patients 2, 3, 4, 5, 6, 9, 10, 13 were confirmed to have no family history, while the family history information for the remaining patients was not accessible.

Subsequent to a thorough filtering process, notable variants were detected in several genes of interest: *APC*, *BRCA2*, *PTCH1*, *PTCH2*, *ELP1* and *SUFU*. In contrast, our investigation did not yield any significant variants in the *GPR161*, *CTNNB1*, *TP53* and *SMARCA4* genes. The distribution of these identified variants is depicted in an accompanying figure (*Figure 3*), revealing a notable disparity in variant distribution across the molecular subgroups. Specifically, the WNT subtype displayed no variants, while the G3, G4, and SHH subgroups had 3, 5, and 6 variants respectively. A striking aspect of our findings is that within these 14 identified variants, each patient exhibited a unique variant, with no overlap. The subtype-specific distribution of these variants was particularly evident: the SHH subgroup exclusively harbored 3 *PTCH1*, 2 *ELP1* and 1 *SUFU* variant, whereas 5 *APC*, 2 *PTCH2* and 1 *BRCA2* variant were found within the G3 and G4 subgroups.

Delving further into the patient clinical subtypes, 4 patients were classified as having classic medulloblastoma (CMB). Among these 4 patients, two patients (patient 9, patient 13) show an SHH subtype with *ELP1* and *PTCH1* gene variants respectively, while the other two patients (patient 1, patient 6) have an *APC* and *BRCA2* variants respectively. Additionally, 5 patients (number 2, 4, 10, 11, 14) were diagnosed with desmoplastic/nodular medulloblastoma (DNMB), comprising three SHH, one G3 and one G4 subtype. Noticeably, only one patient, patient 5, is identified as large cell/anaplastic medulloblastoma (LCAMB) from G4 cohort. The remaining four patients (number 3, 7, 8, 12) lacked histological diagnoses.

Copy number variation (CNV) relation to variant gene

Among the 14 patients, 9 (64%) were detected with CNVs. For example, 1 out of 5 samples with an *APC* gene variant exhibited a deletion in the 5q region, where the gene is located. Additionally, a patient with a *SUFU*

Table 2 Detailed description of the 14 germline variants identified

Patient _ID	Gene	Transcript	Mutant name	Amino acid change	Exon/Intron ID	Zygous	Chr:por:mut	gnomad_EAS_AF	Functional change	In silico: SIFT;LRT; MutTaster;FATHMM	ClinVar classification	Metastasis	Molecular subtype	Recurrence	CNV
1	*APC*	NM_001127511	c.A3056G	p.N1019S	Exon 14	Het	chr5:112838704:A>G	0	Missense	D;D;D;D	Uncertain significance	M0	G3	No	1q+, 3+, 5q-, 6+, 8+, 9+, 10+, 11+, 13+, 15-, i17q, 18+, 19+, 20+, 21+
2	*APC*	NM_001127511	c.G822C	p.L274F	Exon 7	Het	chr5:112815536:G>C	0	Missense	T;N;N;D	Uncertain significance	M2	G3	No	7+, 8-, 10q-, 11, -13, i17q
3	*APC*	NM_001127511	c.A8042G	p.D2681G	Exon 14	Het	chr5:112843690:A>G	4.0620000000000002E−6	Missense	T;N;D;D	Uncertain significance	M0	G3	No	1q+ 8+ 17+
4	*APC*	NM_001127511	c.A1G	p.M1*	Exon 1	Het	chr5:112707718:A>G	5.3090000000000002E−5	Startloss	D;.;D;D	Uncertain significance	M0	G4	No	11p- 17i 22+
5	*APC*	NM_001127511	c.C3247A	p.P1083T	Exon 14	Het	chr5:112838895:C>A	0	Missense	D;D;D;D	Uncertain significance	M0	G4	No	N/A
6	*BRCA2*	NM_000059	c.G7488C	p.K2496N	Exon 15	Het	chr13:32356480:G>C	1.6249999999999999E−5	Missense	D;N;N;D	Uncertain significance	M0	G4	No	N/A
7	*PTCH2*	NM_001166292	c.C1573T	p.R525X	Exon 12	Het	chr1:44828523:G>A	1.223e−05	Nonsense	.;D;A;.	Pathogenic	M2	G4	Yes	N/A
8	*PTCH2*	NM_001166292	c.C1573T	p.R525X	Exon 12	Het	chr1:44828523:G>A	1.223e−05	Nonsense	.;D;A;.	Pathogenic	N/A	G4	N/A	N/A
9	*ELP1*	NM_003640	c.T2A	p.M1*	Exon 2	Het	chr9:108931145:A>T	0	Startloss	D;D;D;T	Uncertain significance	M0	SHH	N/A	9p+, 9q-
10	*ELP1*	NM_001318360	c.C583T	p.Q195X	Exon 10	Het	chr9:108916237:G>A	4.061e−06	Nonsense	.;N;A;.	Pathogenic	M0	SHH	N/A	1q+, 9q-, 20i
11	*PTCH1*	NM_001354918	c.G1370T	p.G457V	Exon 10	Het	chr9:95476835:C>A	0	Nonsense	D;D;D;D	Pathogenic	M0	SHH	Yes	-9
12	*PTCH1*	NM_001354918	c.C2066T	p.A689V	Exon 13	Het	chr9:95481986:C>A	0	Missense	.;D;A;.	Pathogenic	M0	SHH	No	N/A
13	*PTCH1*	NM_000264	c.C529T	p.Q177X	Exon 3	Het	chr9:95485740:G>A	0	Nonsense	.;D;A;.	Pathogenic	N/A	SHH	N/A	No CNV detected
14	*SUFU*	NM_001178133	c.T833C	p.L278P	Exon 7	Het	chr10:102597216:T>C	0	Missense	D;D;D;T	Uncertain significance	M0	SHH	No	2+, 3-, 6+, 10q-, 11p-, 16-, 21+

*, indicates a start codon mutation. ., indicates that no prediction result is available from the respective tool (SIFT, LRT, MutTaster, FATHMM). SHH, Sonic Hedgehog; G3, Group 3; G4, Group 4; Het, heterozygous; Chr, Chromosome; por, position; mut, mutation; gnomad, Genome Aggregation Database; EAS_AF, East Asian Allele Frequency; SIFT, Sorting Intolerant From Tolerant; LRT, likelihood ratio test; MutTaster, mutation taster; FATHMM, functional analysis through hidden Markov models; M0, absence of metastasis; M2, presence of metastasis; CNV, copy number variation; N/A, not available. SIFT: T = tolerated; D = deleterious. LRT: N = neutral; D = deleterious. MutationTaster: D = disease-causing; A = polymorphism (non-disease-causing). FATHMM: T = tolerated; D = damaging.

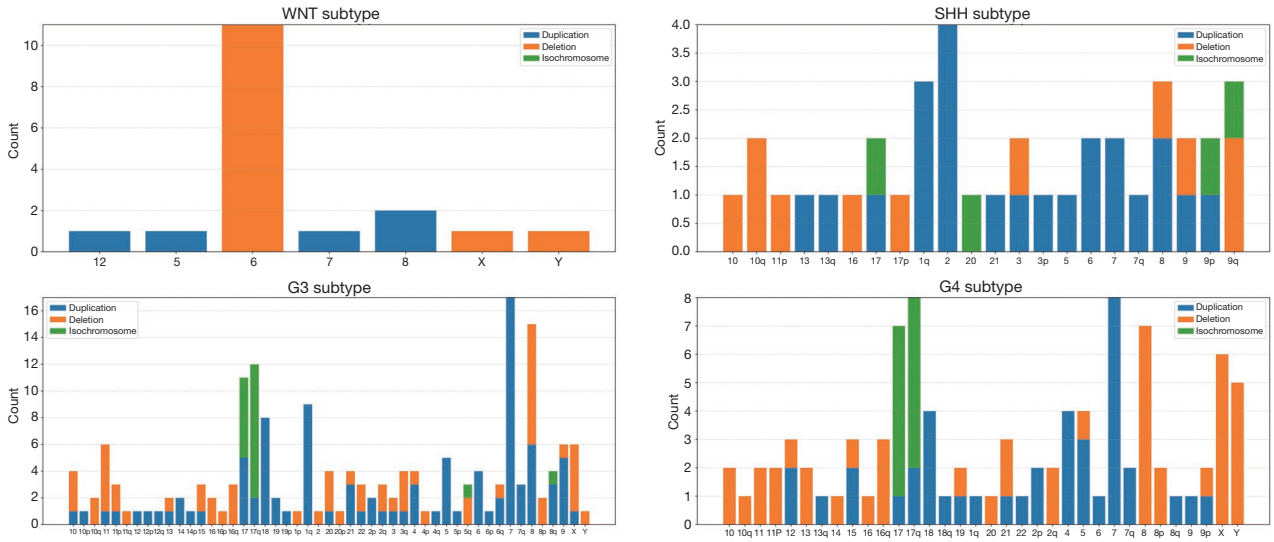

Figure 4 CNV distribution among all subtypes. WNT, Wingless (medulloblastoma subtype); SHH, Sonic Hedgehog (medulloblastoma subtype); G3, Group 3 (medulloblastoma subtype); G4, Group 4 (medulloblastoma subtype); CNV types: duplication, deletion, and isochromosome represent different types of genetic copy number changes. CNV, copy number variation.

variant showed a deletion in the 10q region, and both patients with *ELP1* gene mutations had CNVs in the 9q region. Detailed information on these CNVs and their associations with gene variants can be found in *Table 2*. The distribution of CNVs and molecular subtypes is shown in *Figure 4*. The WNT subtype has the least number of CNVs among all 4 subtypes. G3 has the highest number of CNV occurrences. Noticeably, chromosome 7 duplication occurs in all 4 subtypes, with both G3 and G4 having a high chance of chromosome 7 duplication, while the WNT and SHH subtypes have a lower chance. 17q duplication and isochromosome variants are common in all three subtypes except WNT. In our cohort, some typical CNVs in each subtype were found. The WNT subtype shows a high occurrence of chromosome 6 deletions, while chromosome 2 duplication is solely found in the SHH subtype and comprises a large share of the total CNVs. G3 and G4 share common CNV characteristics, such as a high level of 17 and 17q duplication and deletion, a high level of chromosome 7 duplication, and deletion of chromosome 8. However, G4 has noticeable X and Y deletions compared to G3, WNT, and SHH at the same time. This finding corresponds with current reports on G3 and G4 (9).

Discussion

In our cohort of 113 MB patients, 14 germline variants were identified, of which only 2 had been reported in previous studies. All 14 germline variants are rare across all ethnicities, with a minor allele frequency (MAF) of ≤0.0001. This discovery is the first to reveal the rarity of these variants in the Asian population, providing new insights into their potential impact in specific ethnic groups.

We identified six germline variants labeled as pathogenic in the ClinVar database, including two *PTCH2* c.C1573T (p.R525X), *ELP1* c.C583T (p.Q195X), *PTCH1* c.C529T (p.Q177X), *PTCH1* c.C2066T (p.A689V), and *PTCH1* c.G1370T (p.G457V). Additionally, eight variants were classified as of uncertain significance, including *SUFU* c.T833C (p.L278P), *APC* c.A1G (p.M1V), *APC* c.C3247A (p.P1083T), *APC* c.A3056G (p.N1019S), *APC* c.G822C (p.L274F), *APC* c.A8042G (p.D2681G), *ELP1* c.T2A (p.M1K), and *BRCA2* c.G7488C (p.K2496N). These findings suggest that these pathogenic variants may play a significant pathological role in different subtypes of MB, particularly providing new genetic markers for the Asian population.

Although *APC* gene germline variants are marked as uncertain significance in ClinVar, they show deleterious effects in the SIFT, LRT, MutationTaster, and FATHMM databases, especially in the G3 subtype. This discovery is the first to reveal the potential pathogenic role of *APC* variants in the G3 subtype of MB, highlighting the critical role of this gene in specific subtypes.

In familial study, the *APC* c.G822C (p.L274F) variant is associated with favorable patient outcomes (20).

In our cohort, patients carrying this variant did not experience recurrence and showed good survival rates after radiotherapy and chemotherapy. This finding aligns with existing familial studies and is the first validation in the Asian population of the specific conditions under which *APC* variants may be associated with favorable outcomes.

The only *BRCA2* germline variant, *BRCA2* c.G7488C (p.K2496N), found in our study was classified as of uncertain significance in ClinVar but showed deleterious effects in multiple prediction databases. Although previous study indicated that biallelic *BRCA2* variants are associated with poor prognosis in MB (21), our study is the first to suggest that a single-allele variant may be associated with a relatively good prognosis, particularly in G4 subtype patients.

Although *PTCH2* gene germline variants in the SHH subtype are less reported in the literature, our study found that they are associated with poor prognosis in the G4 subtype. This discovery reveals the potential importance of *PTCH2* in non-WNT/SHH subtypes, suggesting the need for further research on this gene's role in different subtypes.

We first discovered that *ELP1* gene germline variants exist in the SHH subtype and are associated with a 9q arm loss. This finding is consistent with recent research (9,14), further validating the potential pathological role of *ELP1* in SHH subtype MB and providing a new direction for future functional studies of this gene.

Our CNV analysis revealed significant differences between subtypes. G3 and G4 subtypes exhibited higher frequencies of 17q duplications and X chromosome deletions, while chromosome 2 duplications were observed in the SHH subtype for the first time. Additionally, common CNVs in the G3 subtype (such as 7+, 1q+, 17q+, etc.) were observed in our cohort. These CNV patterns not only align with existing research but also, for the first time, reveal the unique genetic characteristics of different subtypes in the Asian population, providing new clues for future research.

This study is the first to provide a detailed subtype-based germline variant landscape specific to the Asian population. Our findings reinforce the connection between *SUFU*, *PTCH1*, and the SHH subtype of MB and confirm, for the first time, the presence of ELP1-related cases in this subtype. The critical role of these genes in the Sonic Hedgehog pathway (22), as well as the potential association between *APC* variants and the G3 and G4 subtypes, provide valuable resources for further investigation.

However, this study has several limitations. Firstly, the sample size was relatively small, which may limit the robustness of the statistical results and the generalizability of the conclusions. Secondly, as all samples were derived from an Asian cohort, the findings may not be fully applicable to other ethnic groups or regions. Additionally, the sequencing and analytical methods employed in this study are subject to certain technical biases, which could potentially impact the accuracy of the results. Future studies should aim to verify these findings using more precise techniques. Lastly, certain confounding factors, such as patients' environmental exposures and lifestyle, were not comprehensively considered in this study, which may have influenced the development and progression of the tumors. Based on these limitations, further large-scale, multicenter studies are warranted to validate our findings and explore additional underlying biological mechanisms.

Conclusions

This study identified 14 rare germline variants in MB patients from an Asian cohort, providing the first detailed subtype-based germline variant landscape for this population. Of these variants, six were classified as pathogenic in ClinVar, and eight were of uncertain significance. The association of *APC* variants with G3/G4 subtypes, along with the involvement of *PTCH1*, *SUFU*, and *ELP1* in the SHH subtype, provides valuable genetic insights into MB. These findings highlight the importance of population-specific genetic research in uncovering novel therapeutic targets and improving treatment strategies. Future research should further investigate the clinical significance of these variants to guide precision medicine approaches in MB.

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Footnote

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1350/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1350/coif). All authors report that they are employed by BGI Research. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The Institutional Review Board of BGI (BGI-IRB 21161-T1). Informed consent was taken from all individual participants or their parents or legal guardians.

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