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Original Article

Somatic mutational profiling and clinical impact of driver genes in Latin-Iberian medulloblastomas: Towards precision medicine

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Medulloblastoma (MB) is the most prevalent malignant brain tumor in children, known for its heterogeneity and treatment-associated toxicity, and there is a critical need for new therapeutic targets. We analyzed the somatic mutation profile of 15 driver genes in 69 Latin-Iberian molecularly characterized medulloblastomas using the Illumina TruSight Tumor 15 panel. We classified the variants based on their clinical impact and oncogenicity. Among the patients, 66.7% were MB_{SHH}, 13.0% MB_{WNT}, 7.3% MB_{Grp3}, and 13.0% MB_{Grp4}. Among the 63 variants found, 54% were classified as Tier I/II and 31.7% as oncogenic/likely oncogenic. We observed 33.3% of cases harboring at least one mutation. *TP53* (23.2%, 16/69) was the most mutated gene, followed by *PIK3CA* (5.8%, 4/69), *KIT* (4.3%, 3/69), *PDGFRA* (2.9%, 2/69), *EGFR* (1.4%, 1/69), *ERBB2* (1.4%, 1/69), and *NRAS* (1.4%, 1/69). Approximately 41% of MB_{SHH} tumors exhibited mutations, *TP53* (32.6%) being the most frequently mutated gene. Tier I/II and oncogenic/likely oncogenic *TP53* variants were associated with relapse, progression, and lower survival rates. Potentially actionable variants in the *PIK3CA* and *KIT* genes were identified. Latin-Iberian medulloblastomas, particularly the MB_{SHH}, exhibit higher mutation frequencies than other populations. We corroborate the *TP53* mutation status as an important prognostic factor, while *PIK3CA* and *KIT* are potential therapeutic targets.

Key words: medulloblastoma, mutational profile, next-generation sequencing, precision medicine, somatic variants.

INTRODUCTION

Brain tumors have a high global mortality rate, and according to the Global Cancer Observatory (GLOBOCAN), they accounted for over 250 000 deaths in 2020.[1] Medulloblastoma (MB) is the most common malignant brain tumor in children and can also occur in adults. It originates embryonically and primarily affects the cerebellum.[2,3] Histologically, medulloblastoma can be categorized into four subtypes: classic, nodular/desmoplastic, extensive nodularity, and large cells/anaplastic.[4] The newest World Health Organization (WHO) central nervous system (CNS) classification divided medulloblastoma into the molecular subgroups that significantly affect patient prognosis: MB_{WNT}, MB_{SHH} *TP53*-mutant, MB_{SHH} *TP53*-wildtype, and MB non-WNT/non-SHH (MB_{Grp3} and MB_{Grp4}).[2,3,5] Since 2017, medulloblastomas have been

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classified into additional subgroups based on their methylation profile, highlighting this tumor's extensive complexity and heterogeneity.[6,7]

The treatment of medulloblastoma primarily involves surgical resection, chemotherapy with multiple drugs, and radiotherapy.[8,9] It is important to note that current treatments can be highly toxic and lead to various sequelae, especially in children who are still in their developmental stages.[9,10] Additionally, some patients might develop resistance to the administered drugs, and relapse might occur, further complicating the clinical management of this type of cancer.[11–13]

Therefore, identifying new therapeutic targets that benefit these patients is urgently needed. Next-generation sequencing (NGS), particularly panels of driver genes that can identify genetic mutations of targeted genes, is routinely used for personalized medicine and demonstrates improvements in the clinical management of patients with brain tumors.[14,15] The mutation profile of medulloblastomas has been highly explored in the North American population.[16] However, its landscape must be better understood in other populations, to diminished the disparity in our genomic knowledge.

This study aimed to characterize the somatic mutational profile of Latin-Iberian medulloblastomas by evaluating somatic variants in 15 driver genes for solid tumors using NGS.

MATERIALS AND METHODS

Patients

This retrospective study evaluated primary and treatment-naïve medulloblastomas between 2000 and 2022 from five institutions in three Latin-Iberian countries: Brazil (Barretos Cancer Hospital, Ribeirão Preto Medical School and Federal University of São Paulo), Argentina (Italian Hospital of Buenos Aires), and Portugal (São João Hospital). Experienced neuropathologists reviewed the cases. The cases were previously characterized into MB_{WNT}, MB_{SHH}, MB_{Grp3}, and MB_{Grp4},[17] and the somatic mutation analysis of *CTNNB1* was performed in MB_{WNT} cases.[18] Furthermore, following the WHO classification,[5] all MB_{SHH}, were evaluated for *TP53* mutation status. Therefore, the present series has an overrepresentation of MB_{SHH} cases. The study was approved by the Research Ethics Committee of Barretos Cancer Hospital (protocol number 1248/2016).

Next-generation sequencing

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue using the commercial QIAamp DNA micro kit (QIAGEN, Germany), as previously described.[19] DNA quality was evaluated by Nanodrop 2000 (Thermo Scientific, USA) and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) was used to assess the DNA concentration with a Qubit dsDNA HS assay kit.

The mutational profile of tumor DNA from 69 patients was determined by next-generation sequencing using the TruSight Tumor 15 (TST15) commercial panel (Illumina, USA) following the manufacturer's instructions and as previously reported.[20]

The TST15 panel evaluates 250 amplicons from 15 genes associated with solid tumors, namely *AKT1*, *BRAF*, *EGFR*, *ERBB2*, *FOXL2*, *GNA11*, *GNAQ*, *KIT*, *KRAS*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA*, *RET*, and *TP53*. Library preparation with the TruSight Tumor 15 was performed before sequencing at the MiSeq platform. SOPHiA DDM software (SOPHia GENETICS, Switzerland) was used for variant analysis. We considered a variant allele frequency (VAF) ≥ 10% and a read depth ≥500×. Additionally, we applied filters to exclude synonymous and intronic variants. The Integrative Genomics Viewer (IGV) allowed the visual inspection of these data.

Classification of genetic variants

The scoring system proposed by the Clinical Genome Resource (ClinGen) and other consortia was used for variant classification.[21] The variants were then classified based on their oncogenicity considering several pieces of evidence available in the databases: Franklin, VarSome; varSEAK; ClinVar; LitVar; cBioPortal; Cancer Genome Interpreter (CGI); Cancer Hotspots; EAP53 server; The *TP53* database; The Genome Aggregation Database (gnomAD); and ABraOM. The guidelines proposed by the Association for Molecular Pathology in conjunction with the American Society of Clinical Oncology and the College of American Pathologists were used to classify the variants into four tiers according to their level of clinical significance.[22] For Tier I classification, we considered the presence of drugs specifically targeting medulloblastomas and/or *TP53* mutations in MB_{SHH}. For Tier II, we included variants associated with drugs currently under investigation for medulloblastomas or previously approved by the Food and Drug Administration (FDA) for other diseases. We also determined which of the identified variants were actionable with the assistance of the OncoKB website (https://www.oncokb.org/).[23]

Clinicopathological characteristics and statistical analyses

Patients' clinicopathological features were collected from patient's clinical files and stored on the REDCap platform. Statistical analyses were conducted using the IBM SPSS Statistics for Windows (version 25), with a significance level of 95% (P-value <0.05). The log-rank and Kaplan–Meier tests were used for overall survival analysis, while Fisher's exact and χ^2 tests were used for clinical–molecular associations.

RESULTS

Patient clinicopathological features

The patients' clinicopathological features are summarized in Table 1. Overall, the median age of medulloblastomas was approximately 12 years, and molecularly, most cases belonged to the Sonic Hedgehog (SHH) molecular subgroup (66.7%, 46/69), followed by Wingless (WNT) (13%, 9/69), Group 4 (13%, 9/69), and Group 3 (7.3%, 5/69) (Table 1).

Mutational profile and actionable variants

The TruSight Tumor 15 panel showed the presence of 63 variants (Table S1). We observed variants in all genes except *AKT1*, *GNAQ*, and *RET*. Of the identified variants, 54% were classified as Tier I or Tier II (34/63) and 31.7% as oncogenic or likely oncogenic (20/63). All oncogenic/likely oncogenic variants were classified as Tier I or Tier II, except for the p.(Glu132Lys) variant in the *NRAS* gene, which was classified as Tier III. Only variables with significant impact (Tier I/II or oncogenic/likely oncogenic) were considered for further analysis.

Overall, we observed that 33.3% (23/69) of cases harbored at least one mutation (Fig. 1). *TP53* (23.2%, 16/69) was the most mutated gene, followed by *PIK3CA* (5.8%, 4/69), *KIT* (4.3%, 3/69), *PDGFRA* (2.9%, 2/69), *EGFR* (1.4%, 1/69), *ERBB2* (1.4%, 1/69), and *NRAS* (1.4%, 1/69) (Fig. 1 and Figure S2).

Tumors of the MB_{SHH} subtype had a higher proportion (41.3%, 19/46) of mutated cases (Fig. 1). The most mutated was the *TP53* gene (32.6%, 15/46), followed by *PIK3CA* (6.5%, 3/46), *KIT*, and *PDGFRA*, with 4.3% (2/46) each, and *NRAS* (2.2%, 1/46). In the MB_{WNT} subgroup, 33.3% of cases (3/9) showed variants (Fig. 1). Mutations in MB_{WNT} were found in *TP53* (11.1%, 1/9), *PIK3CA* (11.1%, 1/9), *KIT* (11.1%, 1/9), and *EGFR* (11.1%, 1/9) genes. Mutations of the *CTNNB1* gene were previously reported in 77.8% (7/9) of the cases.[18] No variant was observed in MB_{Grp3}, and one variant (11.1%, 1/9) in the *ERBB2* was found in MB_{Grp4} (Fig. 1).

Among the 63 identified variants, four were considered therapeutically actionable genes: on *PIK3CA*, p.(Glu545Asp), p.(His1047Arg), and p.(Glu542Lys); and at *KIT*, p.(Arg634Trp).

Table 1 Clinicopathological features of medulloblastomas ($n = 69$)

Characteristics	Parameters	*N*	%
Age at diagnosis	Infant (<4 years)	17	24.6
	Pediatric (4–18 years)	29	42.1
	Adult (>18 years)	23	33.3
Country of origin	Brazil	52	75.4
	Argentina	8	11.6
	Portugal	9	13
Sex	Male	41	59.4
	Female	28	40.6
Molecular subgroup	SHH	46	66.7
	WNT	9	13
	Group 3	5	7.3
	Group 4	9	13
Histology	Classic	31	44.9
	Nodular/desmoplastic	19	27.5
	Extensive nodularity	5	7.2
	Large cell/anaplastic	7	10.2
	NI	7	10.2
Metastasis at diagnosis	Yes	17	24.6
	No	49	71
	NI	3	4.4
Surgical extension	Partial	21	30.4
	Total	41	59.4
	NI	7	10.2
Chemotherapy	Yes	52	75.4
	No	7	10.1
	NI	10	14.5
Radiotherapy	Yes	46	66.7
	No	14	20.3
	NI	9	13
Relapse	Yes	11	15.9
	No	36	52.2
	NI	22	31.9
Progression	Yes	14	20.3
	No	35	50.7
	NI	20	29
Status	Alive	42	60.9
	Death cancer specific	21	30.4
	Death external causes	4	5.8
	NI	2	2.9

Abbreviations: NI, no information; SHH, Sonic Hedgehog; WNT, Wingless.

Association of molecular profile with clinical pathological features and outcome

The Tier I/II or oncogenic/likely oncogenic *TP53* variants were associated with relapse ($P = 0.011$), progression ($P = 0.007$), and lower overall survival ($P = 0.040$) (Fig. 2, Table 2). The low number of cases with a mutation in the other genes hampered further statistical analysis.

No statistically significant difference in survival time was observed for the variables: age at diagnosis, country of origin, gender, molecular subgroup, histological subtype, metastasis, extent of surgery, radiotherapy, and relapse. However, as expected, cases with disease progression showed lower survival ($P = 0.016$). The use of chemotherapy was also statistically significant ($P < 0.001$) (Table 2).

DISCUSSION

The present study evaluated the somatic mutation profile of hotspot regions in 15 driver genes in Latin-Iberian primary medulloblastomas. We observed a higher frequency

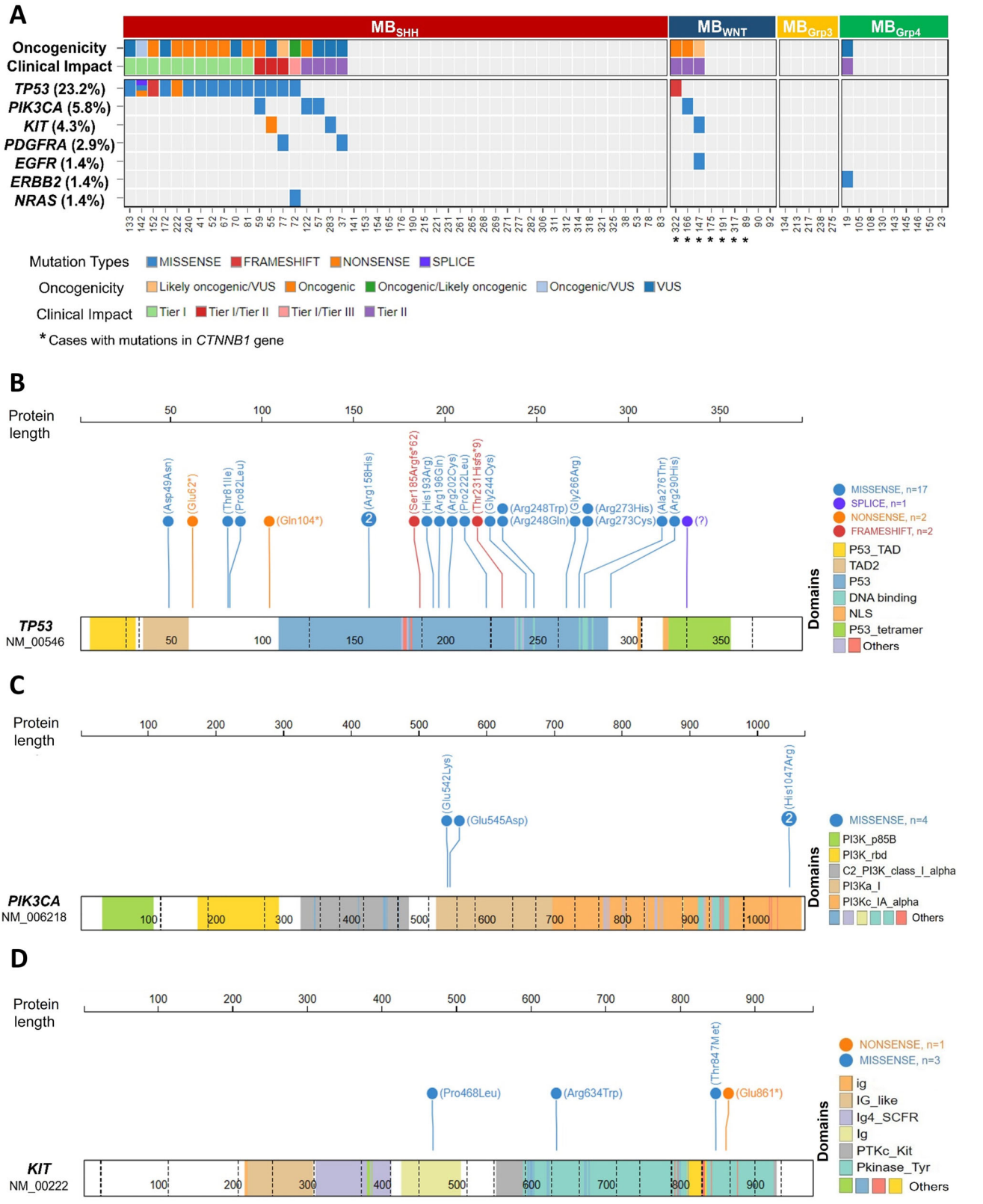

Fig 1 (A) Heatmap of the frequencies of mutated cases with variants classified as Tier I/II or oncogenic/likely oncogenic in the 69 medulloblastomas. (B–D) Lollipop of the identified variants in the three most mutated genes: *TP53*, *PIK3CA*, and *KIT*, respectively.

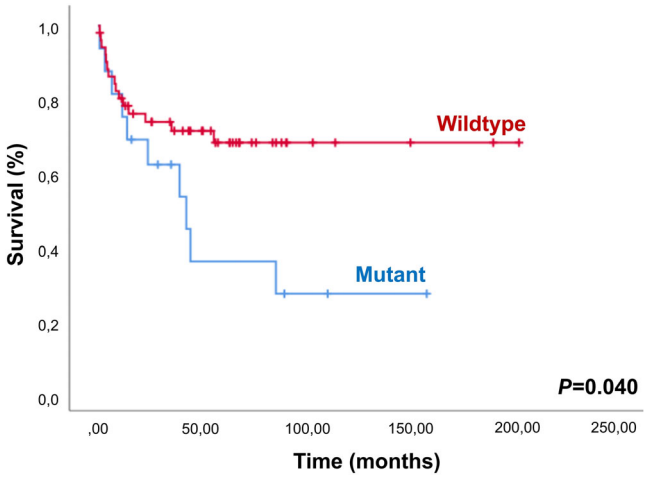

Fig 2 Overall survival of patients with *TP53* variants classified as Tier I/II or oncogenic/likely oncogenic versus wild-type patients.

than reported in other populations. We also found that *TP53* is a prognostic factor, and potential therapeutic variants were identified in the *PIK3CA* and *KIT* oncogenes.

The three most frequently mutated genes were *TP53* (23.2%, 16/69), *PIK3CA* (5.8%, 4/69), and *KIT* (4.3%, 3/69). Interestingly, the frequencies of mutated cases for each gene were higher than reported in the literature. In the cBioPortal database, which includes mainly patients from Europe or North America,[16,24–27] the reported frequencies are: *TP53* (3.9%, 25/645); *PIK3CA* (2.2%, 14/645); and *KIT* (0%, 0/645). In a Chinese study, Wong *et al.* observed the following values for adult medulloblastoma: *TP53* (15.7%, 11/70); *PIK3CA* (4.2%, 3/70); *KIT* (8.6%, 6/70).[28] Moreover, despite our series having an enrichment of the SHH subgroup due to the selection criteria, we observed a higher frequency of *TP53* mutated cases (33%, 15/46) in this subgroup compared with the SHH cases reported by Northcott *et al.* (13% 17/131).[16] There could be several reasons for these differences, including the distinct NGS methodologies or ethnicities. In 2023, reported on the distinct incidence of *CTNNB1* mutations in WNT medulloblastomas.[18]

We identified actionable variants in the *PIK3CA* and *KIT* genes. Three *PIK3CA* variants, p.(Glu545Asp), p.(His1047Arg), and p.(Glu542Lys), are currently

Table 2 Estimate overall survival (5 years) for the clinical and molecular variables

					CI (95%)			CI (95%)		
Variable	Parameters	*N*	OS five years	X̄	Lower	Upper	M_d	Lower	Upper	*P*-value
Age at diagnosis	Infant (<4 years)	17	52.94%	107.15	59.59	154.71	39.36	-	-	0.121
	Pediatric (4–18 years)	28	78.57%	138.20	103.46	172.94	*	-	-	
	Adult (>18 years)	22	54.55%	81.03	46.62	115.43	55.95	8.61	103.29	
Country of origin	Brazil	50	58%	110.16	84.22	136.10	*	-	-	0.143
	Argentina	8	62.5%	54.07	23.80	84.34	35.48	-	-	
	Portugal	9	100%	180.01	138.80	221.23	*	-	-	
Sex	Male	39	58.97%	110.67	78.40	142.93	85.97	-	-	0.195
	Female	28	71.43%	136.63	105.03	168.22	*	-	-	
Molecular subgroup	SHH	45	60%	90.40	67.75	113.06	85.97	-	-	0.329
	WNT	9	88.89%	75.91	60.21	91.60	*	-	-	
	Group 3	5	80%	155.64	74.36	236.91	*	-	-	
	Group 4	8	50%	100.07	36.85	163.28	22.86	-	-	
Histology	Classic	30	66.67%	137.45	104.19	170.70	*	-	-	0.713
	Nodular/Desmoplastic	19	57.89%	92.68	58.41	126.94	*	-	-	
	Extensive nodularity	5	80%	54.01	31.72	76.30	*	-	-	
	Large cells/anaplastic	7	57.14%	38.68	25.17	52.20	44.61	29.74	59.48	
Metastasis at diagnosis	Yes	16	56.25%	102.44	55.13	149.75	42.64	-	-	0.572
	No	48	66.67%	101.25	79.29	123.21	*	-	-	
Surgical extension	Partial	21	47.62%	96.26	53.58	138.94	35.48	0	87.80	0.155
	Total	40	70%	104.21	79.48	128.95	*	-	-	
Chemotherapy	Yes	52	69.23%	133	105.40	160.59	*	-	-	**<0.001**
	No	7	14.29%	10.49	1.24	19.75	3.78	0	9.43	
Radiotherapy	Yes	46	65.22%	124.27	94.22	154.32	*	-	-	0.357
	No	14	57.14%	90.53	49.31	131.74	*	-	-	
Relapse	Yes	11	54.55%	74.02	30.19	117.86	42.64	22.99	62.29	0.846
	No	36	58.33%	113.39	83.45	143.33	*	-	-	
Progression	Yes	14	28.57%	45.39	12.85	77.94	13.99	0	30.84	**0.016**
	No	35	68.57%	129.84	100.15	159.53	*	-	-	
Tier I/II or O/LO	Mutant	16	43.75%	64.85	32.07	97.62	42.64	14.32	70.96	**0.040**
TP53 variants	Wildtype	51	70.59%	142.87	117.24	168.51	*	-	-	

Note: Mean (X̄) and median (Md) values (months) were obtained using the Kaplan–Meier method. * indicates that the median is not reached; the dashes (-) indicate uncalculated limits; bold type represents statistically significant *P*-value ($p < 0.05$). Abbreviations: CI, confidence interval; *N*, number of cases (differs for each variable due to undisclosed data); O/LO: oncogenic/likely oncogenic; OS, overall survival.

indicative of target therapy in metastatic breast cancer, using alpelisib (PI3K inhibitor) and fulvestrant.[29] The present *PIK3CA* variants p.(Glu545Asp), p.(His1047Arg), and p.(Glu542Lys) are considered drivers for triggering oncogenic events by altering the affinity of proteins involved in the PI3K-AKT-mTOR pathway.[30] In medulloblastoma, these variants have been described as more common in the WNT and SHH subgroups, consistent with our findings.[31] Of note, Niesen *et al.* showed that in transgenic mice, *Pik3ca* mutations alone are insufficient to cause developmental alterations or to initiate MB, yet in conjugation with SMO and PTCH1, it results in accelerated tumor SHH MB growth and metastatic capacity.[31] This PI3K-AKT-mTOR pathway pathway can activate GLI non-canonically, promoting the Hedgehog pathway's activation.[32] Therefore, targeted therapies focusing on *PIK3CA* represent a treatment alternative for medulloblastomas, especially for tumors resistant to smoothened (SMO) inhibitors.[30,33,34] The *KIT* variant identified p.(Arg634Trp) is located in exon 13. Mutations in this exon is responsive to sunitinib in gastrointestinal stromal tumors (GIST)[35] and other drugs have been used for these patients with variations in the *KIT* gene in general.[36,37] Further studies are warranted to address the response of medulloblastoma patients harboring these actionable variants.

TP53 was the most frequently mutated gene in our series. We identified that the presence of variants classified as Tier I/II or oncogenic/likely oncogenic was associated with reduced survival compared to wild-type patients and with relapse and progression. This reinforces the prognostic value of *TP53* mutations in medulloblastomas. We also observed the presence of a high VAF (≥70%) in some cases, suggesting a putative germline context, such as Li-Fraumeni syndrome.[38,39] Despite the predominance of Brazilian cases in this series, we did not observe the p.R337H founder variant of *TP53*, which is strongly associated with predisposition to Li-Fraumeni tumors.[40–42] Our TST15 NGS panel covers the complete coding sequencing of the *TP53* gene, including the p53 transactivation (TAD) domain of the 337 codon. Therefore, consistent with this finding, previous studies did not report this variant in CNS tumors, except in choroid plexus carcinoma,[43] or reported a low detection frequency.[44,45] Thus, the present study supports that the p.R337H variant of *TP53* does not predispose individuals to medulloblastomas.

Despite the novelty of the findings of our study, it has some limitations, such as the limited number of cases analyzed, particularly of non-SHH subtype, and the limited clinical data available due to the retrospective and multi-institutional nature of the study. Additionally, evaluating a larger number of cases and expanding the analysis to other medulloblastomas cancer driver genes, such as *PTCH1*,[2,34] would help in the development of appropriate medicine for Latin-Iberian medulloblastoma. In this context, our group is conducting a whole-exome sequencing of Brazilian medulloblastoma, which will allow a landscape molecular analysis of this neoplasm.

In conclusion, this is the most extensive study evaluating a panel of driver genes in primary Latin-Iberian medulloblastomas. We observed, particularly in the MB_{SHH}, higher mutation frequencies than in other populations, corroborated the *TP53* mutation status as an important prognostic factor, and identified *PIK3CA* and *KIT* as potential therapeutic targets.

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DISCLOSURE

Dr. Rui Manuel Reis is an Editorial Board member of Neuropathology and a co-author of this article. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication. The other authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol: This study was approved by the Ethics Committee of Barretos Cancer Hospital (protocol no. 1248/2016).

Informed Consent: Patient consent was waived due to the study's retrospective nature.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

Research involving recombinant DNA: N/A.

DATA AVAILABILITY STATEMENT

Upon request to the corresponding author, data will be available.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/neup.12979/suppinfo.

Figure S1. Graphical abstract of the study. FDA, Food and Drug Administration; VUS, variant of uncertain significance.

Figure S2. Localization of variants in other mutated genes: *EGFR* (A); *ERBB2* (B); *NRAS* (C); *PDGFRA* (D).

Table S1. Variants with VAF ≥10% identified in patients with medulloblastoma and classified according to clinical impact and oncogenicity.