

Original Article

Clinical significance of notch receptors, NPR3, and DLL-3 in pediatric and adult medulloblastoma patients

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ABSTRACT

Objectives: This study aimed to investigate the expression of key genes (Notch-1, Notch-2, Notch-3, Notch-4, DLL1, DLL3, and NPR3) in the Notch signaling pathway in medulloblastoma tumors, and explore their correlation with clinicopathological features, including patient survival outcomes.

Material & Methods: A total of 103 histologically confirmed medulloblastoma tumor samples were analyzed using quantitative Real-time polymerase chain reaction (qPCR) to assess the expression levels of the seven Notch pathway genes. The relationship between gene expression and various clinicopathological parameters, including tumor location, patient age, gender, and risk group classification, was examined. Statistical analyses were performed using SPSS software, with p-values ≤ 0.05 considered statistically significant. Progression-free survival (PFS) and overall survival (OS) were also analyzed to determine the clinical impact of gene expression levels.

Results: The study revealed varying levels of upregulation for the Notch pathway genes in medulloblastoma tumors: Notch-1 (38%), Notch-2 (58%), Notch-3 (82%), Notch-4 (33%), DLL1 (69%), DLL3 (71%), and NPR3 (57%). Notably, Notch-3 expression was significantly associated with tumors located in the occipital region ($p=0.04$). A significant inverse correlation was found between high-risk patients and Notch-4 upregulation ($p=0.05$). DLL3 expression showed significant inverse correlations with gender ($p=0.03$) and risk group classification ($p=0.04$), but a positive correlation with the primary tumor site ($p=0.003$). NPR3 expression was inversely correlated with patient age ($p=0.01$). Furthermore, all Notch receptors and ligands demonstrated positive intercorrelations, suggesting a coordinated role in tumor biology. Survival analysis showed that patients in the average-risk group had significantly higher recurrence rates at both 12 and 24 months compared to high-risk patients ($p<0.01$). Notably, patients who received only radiotherapy post-surgery had lower recurrence rates and better survival outcomes, with significantly lower incidences of death at 12 months ($p=0.005$) and 24 months ($p=0.03$).

Conclusion: The findings highlight the potential clinical significance of Notch-3, Notch-4 receptors, and DLL3 as key biomarkers in medulloblastoma. These genes offer promising targets for developing more personalized and effective treatment strategies. Further research into their precise role in tumor progression and response to therapy is warranted.

Keywords: High risk, Medulloblastoma, Notch signalling pathway, OS, PFS

INTRODUCTION

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, arising in the cerebellum. The World Health Organization (WHO) CNS5 classification categorizes MB into four molecular subgroups: WNT-activated, SHH-activated (TP53-wildtype/mutant), and non-WNT/non-SHH (groups 3 and 4).^[1] According to data from The Central Brain Tumor Registry of the United States Statistical Report, the most common MB subtype was Sonic Hedgehog [SHH]-activated & TP53-wildtype [included in the SHH group], followed by Non-WNT/non-SHH MB [including both

group 3/group C and group 4/group D]. Despite advances in multimodal therapy, a subset of MB remains incurable, with survivors often facing long-term cognitive and physiological impairments.^[2,3]

The Notch signaling pathway plays a key role in neural development, regulating proliferation, differentiation, and apoptosis.^[4] Initially identified in *Drosophila*, Notch receptors (Notch-1, Notch-2, Notch-3, Notch-4) and ligands (DLL1, DLL3) influence tumorigenesis in a context-dependent manner, acting as oncogenic drivers in some cancers and tumor suppressors in others. However, its role in MB remains unclear.^[5]

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This study investigates the expression of seven Notch pathway genes, *Notch-1*, *Notch-2*, *Notch-3*, *Notch-4*, *DLL1*, *DLL3*, and *NPR3* in 103 MB formalin-fixed, paraffin-embedded (FFPE) tissue samples using real-time polymerase chain reaction (RT-PCR). Understanding Notch signaling in MB may provide insights into its pathogenesis and therapeutic potential.

MATERIAL & METHODS

Ethics approval and informed consent

This study was reviewed and approved by the Institutional Ethics Committee [Ref# EC-BHR-O-21-2020]. The study obtained informed consent from each individual participant.

Patients

A total of 103 untreated newly diagnosed patients with histologically confirmed MB tumors registered at The Gujarat Cancer & Research Institute, Ahmedabad, India, from January 2015 to September 2022 were enrolled in the current retrospective study. Detailed clinical and pathological history of enrolled patients was obtained from the case files maintained at the Medical Record Department of the institute. The clinical and pathological characteristics of the enrolled patients are listed here. Sixty-eight percent of patients were male, and 80 percent were pediatric. Patients included in this study were those with histologically confirmed MB who were newly diagnosed and had not received any prior treatment. Only patients with complete clinical and pathological records were considered for inclusion. Exclusion criteria encompassed individuals with a history of prior treatment for MB, cases with incomplete clinical or pathological data, and patients diagnosed with other concurrent malignancies.

Patients < 3 years of age, with metastasis at diagnosis, or residual tumors >1.5 cm² in the area are considered high-risk group patients, while patients not meeting these criteria are considered in the average-risk group, as per the established risk stratification guidelines.^[6] Here, 77% [79/103] patients

are in the high-risk group. All patients underwent surgery as primary treatment. Fifty-three percent of patients had taken radiotherapy and/or chemotherapy as adjuvant therapy. Based on the primary tumor site of MB, there are five different sites, including posterior fossa sol, sub-occipital region, occipital, cerebellum region, and 4th ventricle. In the present study, 37% [38/103] of patients' tumors were located at the posterior fossa sol.

RNA extraction and gene expression analysis

FFPE blocks from the same patients were collected from the pathology department of the institute and subjected to ribonucleic acid (RNA) extraction using the High Pure formalin fixed paraffin embedded tissue (FEPET) RNA Isolation Kit [Roche Life Science]. RNA extraction was carried out for RT-PCR analysis of Notch signaling pathway gene expression. Subsequently, the Qubit Fluorometer [Invitrogen, USA] was employed to measure RNA concentrations.

Gene expression of the notch signaling pathway using real-time PCR

For Notch signaling pathway gene expression detection, complementary deoxyribonucleic acid (cDNA) synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit with 1 µg RNA. Rotor-Gene Q 5-plex HRM instrument [Qiagen] was utilized for relative quantification.^[7] Amplification involved QuantiTect SYBR Green RT-PCR Kit, with 0.4 µl each of forward and reverse primers [Table 1] and 10 µl SYBR Green RT-PCR Master Mix in a total 20 µL reaction volume. The protocol included an initial denaturation step at 95°C for 15 minutes, followed by 45 cycles at 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The 18s RNA gene served as a housekeeping gene, and the commercially available normal brain [Takara Bio Inc. US] served as a calibrator. Relative quantification employed the 2-ΔΔCt method, presenting data as a fold change of gene expression.^[8] Here, fold change less than 1 was considered

Table 1: Primer sequences.

Gene	Forward primers	Reverse primers
Notch-1	TGAATGGCGGGAAGTGTGAAG	GGTTGGGGTCCTGGCATCG
Notch-2	GCCACTGTGTGCTGCCATAC	GGGAACAGGGAGCCAATACTG
Notch-3	ATGGTATCTGCACCAACCTGG	GATGTCCTGATCGCAGGAAGG
Notch-4	AACTCCTCCCCAGGAATCTG	CCTCCATCCAGCAGAGGTT
NPR-3	CTAGGAGCTGGCTTGCTAAT	GGGTTCGCCTCTCAATGGTTAT
DLL1	GATGTGATGAGCAGCATGGA	CCATGGAGACAGCCTGGATA
DLL3	AATCGCCCTGAAGATGTAGACC	GCACCACCGAGCAAATACAA
18s RNA	GGAGTATGGTTGCAAAGCTGA	ATCTGTCAATCCTGTCCGTGT

RNA: Ribonucleic acid

as a downregulation of the gene, while values greater than 1 were considered as an upregulation of the gene.

Statistical analysis

Statistical analyses conducted in SPSS version 20.0 included a two-tailed chi-square [χ^2] test for parameter relationships. Correlations with *Notch-1* gene expression were evaluated using Spearman's correlation coefficient [r]. Kaplan-Meier survival analysis evaluated PFS and OS. Multivariate survival analysis utilized the Cox Regression step-wise Forward LR model, with significance set at $P \leq 0.05$.

RESULTS

Gene expression in MB patients

In the present study, we observed seven gene expressions using qPCR. In which we calculate relative gene quantification compared to the normal brain [Figure 1a] and calculate gene expression as fold change value [Figure 1b]. In fold change value < 1 was considered as a downregulation of the gene, and > 1 was upregulation of the gene.

In MB tissue, *Notch-4* is slightly highly expressed compared to normal brain tissue, and *Notch-1* is expressed the same as normal brain expression or all other genes have slight downregulation compared to normal brain in which *Notch-3* has notably decreased expression.

Notch signaling pathway gene expression and clinicopathological parameter correlation

Gene expression of MB tumor as fold change value shows 38%, 58%, 82%, 33%, 69%, 71%, and 57% upregulation in *Notch-1*, *Notch-2*, *Notch-3*, *Notch-4*, *DLL1*, *DLL3*, and *NPR3*, respectively [Figure 1b].

Correlation between *Notch-1* and *Notch-2* gene expression and clinicopathological parameters shows no significant association with any parameters. Whereas *Notch-3* correlation with clinicopathological parameters shows a significantly high correlation with the primary tumor site. Tumors at the occipital site have 100% [09/09] significant upregulation of the *Notch-3* gene compared to other sites. [$\chi^2=10.55$, $r=0.11$, $p=0.04$] and correlation between *Notch-4* gene expression and clinicopathological parameters shows a significant association with risk group and *Notch-4* gene expression. Patients in the high-risk group show 38% upregulation than patients in the average-risk group [$\chi^2=3.77$, $r=-0.19$, $p=0.05$]. In ligand gene expression, a correlation between *DLL3* gene expression and clinicopathological parameters showed a strong significant correlation between gender, risk group, and primary tumor site. Female patients showed 85% upregulation of the gene compared to male patients [$\chi^2=4.59$, $r=-0.21$, $p=0.03$], and patients in the high-risk group showed 76% upregulation of the *DLL3* gene than average group patients [$\chi^2=4.23$, $r=-0.20$, $p=0.04$]. Patients with tumors located at the 4th ventricle region show 90% upregulation of the *DLL3* gene than another tumor site [$\chi^2=10.15$, $r=0.25$, $p=0.003$]. Also, the correlation between *NPR3* gene expression and clinicopathological parameters shows pediatric patients showed 63% upregulation than adults [$\chi^2=6.18$, $r=-0.24$, $p=0.01$] [Figure 2, Table 2].

Inter gene correlation

To evaluate the correlation of the expression patterns among these 7 genes, we performed pairwise Spearman's correlation tests. A strong correlation was detected among all genes and all genes show strong positive associations with each other. A significantly positive correlation was observed between the *Notch-1* gene with *Notch-2* [$\chi^2=19.15$, $r=0.53$, $p=0.001$], *Notch-3* [$\chi^2=10.15$, $r=0.25$, $p=0.01$], *Notch-4* [$\chi^2=13.12$, $r=$

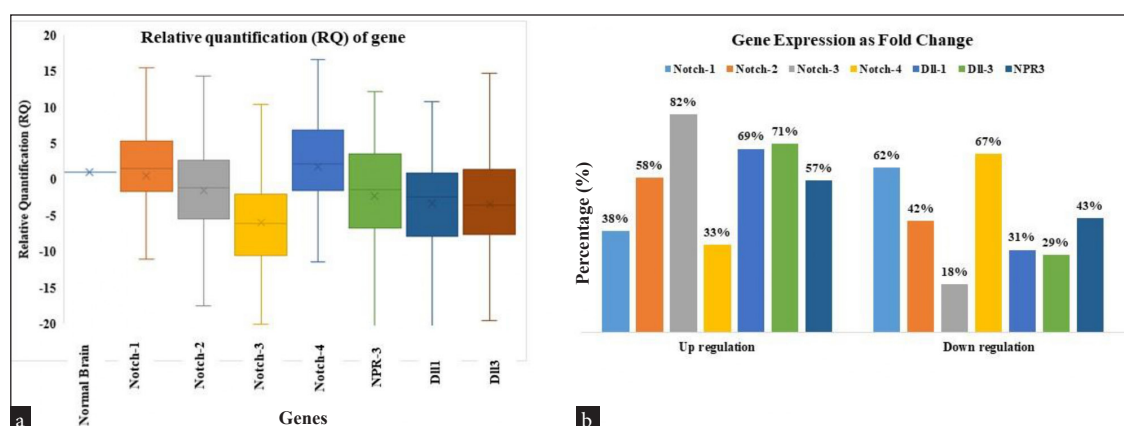


Figure 1: Notch pathway gene expression, (a) The relative quantification value, normalized by the reference normal brain for seven genes, RNA is displayed as a log-transformed number. The upper and lower quartiles are shown by the top and bottom of the box, respectively. The median value for all samples is shown by the band in the center of the box. (b) Gene expression as Fold change.

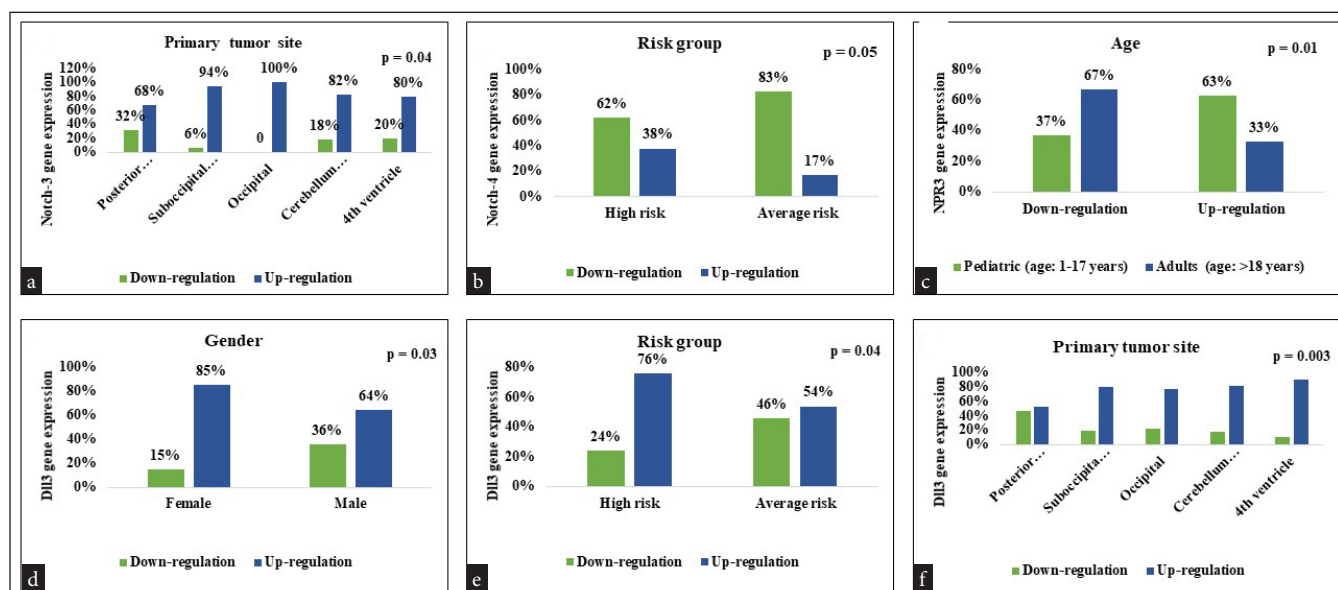


Figure 2: Correlation between clinicopathological parameter and Notch signaling pathway gene (a) Correlation between tumor site and Notch-3 gene expression, (b) Correlation between risk group and Notch-4 gene expression (c) Correlation between age and NPR3 gene expression (d-f) Correlation between gender, risk group, tumor site and DLL3 gene expression.

0.43, $p = 0.001$], *NPR3* [$\chi^2=9.25$, $r = 0.35$, $p = 0.001$], and *DLL-1* [$\chi^2 = 7.50$, $r = 0.26$, $p = 0.007$]. Each receptor and ligand of the Notch signaling pathway showed inter correlations, demonstrating that from ligand to receptors, the pathway is connected and showed significance in MB tumor growth [Table 3].

Univariate survival analysis in MB patients for PFS and OS using Kaplan-Meier analysis

In progression-free survival analysis, for both 12 and 24 months' analysis, patients with an average risk group have a notable incidence of recurrence than high-risk group patients. That shows patients in the average risk group are more likely to have a recurrence [Log rank=40.26, $p < 0.01$] [Log rank=54.36, $p < 0.01$]. In 24 months of PFS analysis, Patients who receive only radiotherapy after surgery have no recurrence compared to patients who receive chemotherapy after surgery [Log rank=8.96, $p=0.03$]. There was no significance noted in any of the genes for 12 or 24 months of PFS analysis. Univariate survival analysis for 12 and 24-month OS demonstrated that patients who receive radiotherapy after surgery have a low incidence of death compared to patients receiving chemotherapy, chemotherapy, and radiotherapy, or only surgery for treatment [Log rank=12.74, $p=0.005$] [Log rank=8.73, $p=0.03$]. According to tumor location, patients with tumors located at the sub-occipital region of the brain show a high incidence of death compared to patients with other tumor locations [Log rank=9.01, $p=0.06$] [Table 4, Figure 3].

DISCUSSION

The observed upregulation of Notch pathway genes in MB tumors suggests their potential involvement in tumor progression. In MB, Notch-1 and Notch-2 have opposite biological effects. Notch-1 inhibits proliferation of MB, whereas Notch-2 promotes cell growth of MB. We found that expression of Notch-1 is not detectable, whereas Notch-2 is overexpressed in MB.^[9] This observation is consistent with previous studies, such as those by Aburjania *et al.*,^[10] which demonstrated that Notch-1 functions as a tumor suppressor in MB, while *Notch-2* acts as an oncogene. The correlation between Notch pathway gene expression and clinicopathological parameters highlights the importance of considering molecular subtypes in MB patient stratification. Notably, the association of *Notch-3* expression with the primary tumor site and *Notch-4* expression with the risk group suggests their potential as prognostic markers.

Notch-3 upregulation has been observed in various cancers, including brain tumors like osteosarcoma, chondrosarcoma, and rhabdomyosarcoma, where it plays a role in embryonic brain tumor growth and glioma development. Notch-3 inhibition enhances the effectiveness of radiotherapy in brain tumors, and its dysregulation has been associated with tumor aggressiveness, maintenance, and resistance to chemotherapy. In osteosarcoma and chondrosarcoma, *Notch-3* is upregulated, particularly in hypercellular areas of chondrosarcoma, suggesting a role in regulating stemness.^[10] Similar findings were reported by Wang *et al.*, who highlighted Notch-3's role in enhancing glioma stem cell properties and radio-resistance.

Table 2: Correlation between Notch signaling pathway gene expression and clinicopathological parameter						
Parameters	N	Notch-1 gene expression		χ^2	r	P-value
	103	Down-regulation N = 64	Up-regulation N = 39			
Tumor type						
Classical type	78	52 (67)	26 (33)	7.62	0.17	0.08
Nodular type	13	07 (54)	06 (46)			
Desmoplastic type	08	03 (37)	05 (63)			
Anaplastic	02	02 (100)	00 (00)			
rhabdoid type	02	00 (00)	02 (100)			
Parameters		Notch-3 gene expression				
		Down-regulation N = 18	Up-regulation N = 85			
Primary tumor site						
Posterior Fossa SOL	38	12 (32)	26 (68)	10.5	0.11	0.04
suboccipital region	35	02 (06)	33 (94)			
Occipital	09	00 (00)	09 (100)			
cerebellum region	11	02 (18)	09 (82)			
4 th ventricle	10	02 (20)	08 (80)			
Parameters		Notch-4 gene expression				
		Down-regulation N = 69	Up-regulation N = 34			
Risk group						
High	79	49 (62)	30 (38)	3.77	-0.19	0.05
Average	24	20 (83)	04 (17)			
Parameters		DLL1 gene expression				
		Down-regulation N = 32	Up-regulation N = 71			
Gender						
Female	33	14 (42)	19 (58)	2.92	0.16	0.08
Male	70	18 (26)	52 (74)			
Parameters		DLL3 gene expression				
		Down-regulation N = 30	Up-regulation N = 73			
Gender						
Female	33	05 (15)	28 (85)	4.59	-0.21	0.03
Male	70	25 (36)	45 (64)			
Risk group						
High	79	19 (24)	60 (76)	4.23	-0.20	0.04
Average	24	11 (46)	13 (54)			
Primary tumor site						
Posterior fossa sol	38	18 (47)	20 (53)	10.15	0.25	0.003
Suboccipital region	35	17 (20)	28 (80)			
Occipital	09	02 (22)	07 (78)			
Cerebellum region	11	02 (18)	09 (82)			
4 th ventricle	10	01 (10)	09 (90)			
Parameters		NPR3 gene expression				
		Down-regulation N = 44	Up-regulation N = 59			
Age						
Pediatric (age: 1-17 years)	82	30 (37)	52 (63)	6.18	-0.24	0.01
Adults (age: >18 years)	21	14 (67)	07 (33)			

Bold P values due to results are significant or nearly significant. SOL: Space occupying lesion

Table 3: Inter gene correlation.

Genes	Notch1	Notch2	Notch3	Notch4	NPR3	DLL1	DLL3
Notch1	-	r = 0.53 P = 0.001	r = 0.25 P = 0.01	r = 0.43 P = 0.001	r = 0.35 P = 0.001	r = 0.26 P = 0.007	r = 0.14 P = 0.13
Notch2	-	-	r = 0.44 P = 0.001	r = 0.55 P = 0.001	r = 0.50 P = 0.001	r = 0.32 P = 0.001	r = 0.28 P = 0.004
Notch3	-	-	-	r = 0.32 P = 0.001	r = 0.22 P = 0.02	r = 0.35 P = 0.001	r = 0.43 P = 0.001
Notch4	-	-	-	-	r = 0.53 P = 0.001	r = 0.47 P = 0.001	r = 0.31 P = 0.001
NPR3	-	-	-	-	-	r = 0.39 P = 0.001	r = 0.22 P = 0.02
DLL1	-	-	-	-	-	-	r = 0.44 P = 0.001
DLL3	-	-	-	-	-	-	-

Bold values are significant results. DLL: Delta like legand, NPR: Natriuretic peptide receptor

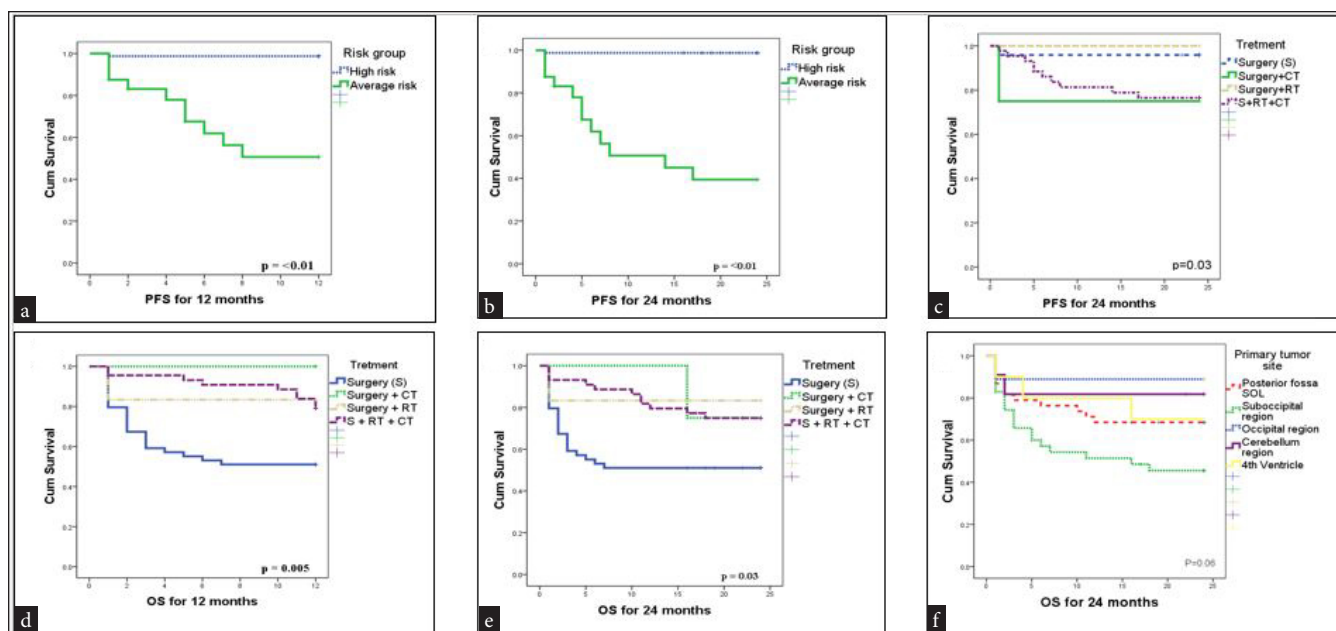


Figure 3: Kaplan-Meier survival curves for 12 and 24 months PFS and OS (a) Patients risk group (12 months PFS), (b) Patients risk group (24 months PFS), (c) Treatment (24 months PFS) (d) Treatment (12 months OS) (e) Treatment (24 months OS), (f) Tumor site (24 months OS). PFS: Progression-free survival, OS: Overall survival

Notch-4 has been implicated in the development of breast cancer,^[11,12] where it has been shown to be overexpressed in a subset of tumors. However, the mechanism by which Notch-4 is upregulated in high-risk patients with brain tumors has not been directly addressed in the results. It is possible that *Notch-4* up-regulation in brain tumors is due to similar mechanisms as those observed in breast cancer, such as Mouse Mammary Tumor Virus (MMTV) integration into the *Notch-4* locus or the activation of oncogenic signaling pathways. Further research, such as those conducted by Nagamatsu *et al.*^[12] on

breast cancer, is needed to determine the specific mechanisms by which *Notch-4* is upregulated in brain tumors of high-risk group patients.

Furthermore, the gender-specific and age-specific differences in *DLL3* and *NPR3* expression underscore the need for personalized treatment approaches in MB patients. *DLL3* is upregulated in female brain tumor patients, specifically in low-grade gliomas and proneural glioblastomas.^[13] The *NPR3* gene has been implicated in various cancers, including pediatric tumors. *NPR3* has been reported to exert a tumor-

Table 4: Univariate survival analysis for PFS and OS using Kaplan-Meier analysis.

Characteristics	N=103	Progression-free survival				Overall survival			
		12 months		24 months		12 months		24 months	
		Disease relapsed	Log-rank p-value	Disease relapsed	Log-rank p-value	Patients died	Log-rank p-value	Patients died	Log-rank p-value
		N (%)		N (%)		N (%)		N (%)	
Age			NS		NS		NS		
Pediatrics (age: 1-17 years)	82	10 (12)		12 (15)		30 (37)		33 (40)	3.05
Adults (age: >18 years)	21	01 (05)		01 (05)		04 (19)		04 (19)	0.08
Gender			NS		NS		NS		NS
Female	33	03 (09)		03 (09)		11 (33)		11 (33)	
Male	70	08 (11)		10 (14)		23 (33)		26 (37)	
Tumor type			NS		NS		NS		NS
Classical type	78	07 (09)		09 (12)		29 (37)		32 (41)	
Nodular type	13	02 (15)		02 (15)		02 (16)		02 (15)	
Desmoplastic type	08	02 (25)		02 (25)		01 (13)		01 (13)	
Anaplastic	02	00 (00)		00 (00)		01 (50)		01 (50)	
Rhabdoid type	02	00 (00)		00 (00)		01 (50)		01 (50)	
Risk group							NS		NS
High	79	01 (01)	40.26	01 (01)	54.36	28 (35)		31 (39)	
Average	24	10 (42)	<0.01	12 (50)	<0.01	06 (25)		06 (25)	
Treatment									
Surgery (S)	49	02 (04)	6.84	02 (04)	8.96	24 (49)	12.74	24 (49)	8.73
Surgery + CT	04	01 (25)	0.07	01 (25)	0.03	00 (00)	0.005	01 (25)	0.03
Surgery + RT	06	00 (00)		00 (00)		01 (17)		01 (17)	
S + RT + CT	44	08 (18)		10 (23)		09 (20)		11 (25)	
Primary tumor site			NS		NS		NS		
Posterior fossa sol	38	04 (11)		05 (13)		12 (32)		12 (32)	9.01
Sub occipital region	35	05 (14)		05 (14)		17 (49)		19 (54)	0.06
Occipital	09	00 (00)		01 (11)		01 (11)		01 (11)	
Cerebellum region	11	01 (09)		01 (09)		02 (18)		02 (18)	
4th ventricle	10	01 (10)		01 (10)		02 (20)		03 (30)	
Notch1 expression			NS		NS		NS		NS
Down-regulated	64	07 (11)		09 (14)		24 (38)		26 (41)	
Up-regulated	39	04 (10)		04 (10)		10 (26)		12 (31)	
Notch2 expression			NS		NS		NS		NS
Down-regulated	43	04 (09)		05 (12)		17 (40)		17 (40)	
Up-regulated	60	07 (12)		08 (13)		17 (28)		21 (35)	
Notch3 expression			NS		NS		NS		NS
Down-regulated	18	02 (11)		02 (11)		06 (33)		06 (33)	
Up-regulated	85	09 (11)		11 (13)		28 (33)		32 (38)	
Notch4 expression			NS		NS		NS		NS
Down-regulated	69	08 (12)		10 (14)		23 (33)		23 (33)	
Up-regulated	34	03 (09)		03 (09)		11 (33)		15 (44)	
DLL1 expression			NS		NS		NS		NS
Down-regulated	32	04 (13)		05 (16)		12 (38)		12 (38)	
Up-regulated	71	07 (10)		08 (11)		22 (31)		26 (37)	
DLL3 expression			NS		NS		NS		NS
Down-regulated	30	05 (17)		06 (20)		12 (40)		12 (40)	
Up-regulated	73	06 (08)		07 (10)		22 (30)		26 (36)	
NPR3 expression			NS		NS		NS		NS
Down-regulated	44	04 (09)		05 (11)		16 (37)		16 (37)	
Up-regulated	59	07 (12)		08 (14)		18 (31)		22 (37)	

NS: No significance, Bold values are significant results. RT: Radio therapy, CT: Chemo therapy

suppressive effect in clear cell renal cell carcinoma and promote the proliferation and migration of triple-negative breast cancer [TNBC] cells and ER+ cells. However, the specific upregulation of the *NPR3* gene in pediatric tumor patients is not directly addressed in the study.^[14]

The analysis of progression-free survival [PFS] in patients with different risk groups and treatment modalities reveals important insights into recurrence and survival outcomes in cancer patients. The results indicate that patients in the average-risk group are more likely to experience recurrence compared to those in the high-risk group, as evidenced by the notable incidence of recurrence at both 12 and 24 months' analysis [Log rank=40.26, $p<0.01$] [Log rank=54.36, $p<0.01$]. This aligns with the findings of LV *et al.*^[14], who developed a clinical score for predicting recurrence in colon cancer patients, demonstrating that higher-risk patients had significantly shorter times to recurrence.

Furthermore, the impact of treatment modalities on recurrence is evident, particularly in the 24-month PFS analysis. Patients who received only radiotherapy after surgery had no recurrence compared to those who received chemotherapy after surgery [Log rank=8.96, $p=0.03$]. This underscores the significance of treatment selection in influencing recurrence outcomes and emphasizes the potential benefits of specific treatment approaches in preventing disease recurrence. Similar studies have shown that patients missing radiation therapy sessions had an increased risk of disease recurrence.^[15] Interestingly, our study did not find any significant associations between gene expression patterns and PFS outcomes at 12 or 24 months. This suggests that factors beyond the analyzed genes may play a more substantial role in determining recurrence risk and survival outcomes, an observation also noted in other studies focusing on MB recurrence and survival.

In terms of OS analysis, the univariate survival analysis for 12 and 24-month OS revealed important findings related to treatment modalities. Patients who received radiotherapy after surgery demonstrated a lower incidence of death compared to those receiving chemotherapy, chemotherapy, and radiotherapy, or only surgery for treatment [Log rank=12.74, $p=0.005$] [Log rank=8.73, $p=0.03$]. This highlights the potential survival benefits associated with specific treatment sequences in cancer management. Studies on radiation therapy have found that mortality rates during radiation therapy are low, except in palliative care cases.^[16] Additionally, research has indicated that acute pneumonitis and chemotherapy-related toxicity contribute to mortality in patients undergoing thoracic radiotherapy.^[17]

Moreover, tumor location significantly impacted patient outcomes. Patients with tumors located in the suboccipital

region of the brain showed a higher incidence of death compared to patients with tumors in other locations [Log rank=9.01, $p=0.06$]. This is consistent with findings that tumors in the suboccipital region can lead to severe complications such as increased intracranial pressure, resulting in neurological deficits and higher mortality rates.

Overall, our findings contribute to a better understanding of the molecular mechanisms underlying MB pathogenesis and may facilitate the development of targeted therapies for improved patient outcomes. This study underscores the complex interplay of risk factors, treatment modalities, and tumor characteristics in influencing recurrence and survival outcomes in cancer patients. Tailoring treatment strategies based on risk stratification, treatment modalities, and tumor characteristics can potentially improve patient outcomes and guide personalized cancer care.

Our study provides Notch pathway gene expression in MB, identifying potential prognostic markers and offering insights into treatment efficacy and recurrence. Additionally, survival analysis contributes valuable insights into treatment efficacy and recurrence patterns in MB patients, which is a strength of this study. However, it lacks functional validation which calls for further research.

CONCLUSION

This study highlights the clinical significance of *Notch-3*, *Notch-4*, *DLL3*, and *NPR3* in medulloblastoma, demonstrating their potential as prognostic biomarkers. *Notch-3* was significantly associated with tumor location, while *Notch-4* and *DLL3* were linked to high-risk patient stratification. Importantly, treatment outcomes varied based on post-surgical therapy. Patients who received only radiotherapy had significantly lower recurrence rates and improved survival compared to those receiving chemotherapy or combined therapy, underscoring the potential of radiotherapy in reducing early mortality. Additionally, the strong intercorrelation among Notch pathway components suggests a coordinated role in tumor progression. These findings emphasize the need for molecular profiling in treatment decisions and warrant further research to optimize therapy combinations, improving patient outcomes while minimizing treatment-related toxicity.

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