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Original Article

The Association of *NSUN6* Gene Polymorphisms with Neuroblastoma Risk in Children from Jiangsu Province: A Case-control Study

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Abstract

Background and objectives: 5-methylcytosine RNA modification is a key regulator of neuroblastoma oncogenesis and differentiation. *NSUN6*, a 5-methylcytosine-specific messenger RNA methyltransferase, modulates messenger RNA methyltransferase activity and translation termination. Yet, its potential link to neuroblastoma risk has not been previously reported. The present study aimed to reveal the relationship between *NSUN6* gene polymorphisms and the risk of neuroblastoma in children from Jiangsu province.

Methods: In this case-control study, we investigated three *NSUN6* gene polymorphisms (rs3740102 A>C, rs12780826 T>A, and rs61842187 G>C) in 402 neuroblastoma cases and 473 controls, all of whom were children from Nanjing City, Jiangsu Province, China. DNA from these subjects was assessed using the TaqMan method. Multivariate logistic regression analysis was employed to examine the association between *NSUN6* gene polymorphisms and neuroblastoma risk. Additionally, the Genotype-Tissue Expression database was utilized to elucidate the impact of these polymorphisms on *NSUN6* and nearby gene expression. Kaplan-Meier analysis and the non-parametric test were conducted on the R2 platform to assess the relationship between gene expression, prognosis, and neuroblastoma risk.

Results: Carriage of two to three protective genotypes (rs3740102 AA/AC, rs12780826 TT/TA, rs61842187 CC) was significantly associated with a lower risk of neuroblastoma (adjusted odds ratio = 0.41, 95% confidence interval = 0.23–0.73, $P = 0.002$), with consistent results across all subgroups. Expression quantitative trait locus analysis showed these single-nucleotide polymorphisms may upregulate the expression of *NSUN6* and *CACNB2*. Furthermore, higher *NSUN6* and *CACNB2* expression was correlated with a potentially lower risk of neuroblastoma, improved overall survival (*NSUN6*: $P = 2.54e-03$; *CACNB2*: $P = 6.35e-06$) and event-free survival (*NSUN6*: $P = 7.90e-04$; *CACNB2*: $P = 4.64e-06$), as well as a lower likelihood of *MYCN* amplification.

Conclusions: *NSUN6* rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes may be associated with a better prognosis of neuroblastoma. This association may be related to the potential upregulation of *NSUN6* gene expression and a lower likelihood of *MYCN* amplification.

Keywords: Neuroblastoma; Polymorphism; *NSUN6*; Susceptibility; Case-control Studies; Jiangsu province.

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Introduction

Neuroblastoma, an embryonic tumor originating from neural crest cells, represents a significant concern in pediatric oncology, being responsible for over 15% of childhood cancer-related fatalities. In

the United States alone, around 650 new cases of neuroblastoma are diagnosed annually, underscoring the urgent need for continued research, early detection methods, and improved treatment options for this challenging disease. However, due to its distinct heterogeneity in genetic, clinical, and morphological aspects, the efficacy of current treatment modalities for neuroblastoma remains limited.[1] Given this complexity, it is imperative to explore safer and more effective treatment approaches grounded in the genetic variations and molecular signatures of neuroblastoma.

5-methylcytosine (m^5C) is a prevalent RNA modification found in many RNA species, including ribosomal RNAs, transfer RNAs (hereinafter referred to as tRNAs), messenger RNAs (mRNAs), enhancer RNAs, and noncoding RNAs, playing a pivotal role in numerous aspects of both nuclear gene and mitochondrial expression. Its functions encompass RNA stability, export, ribosome assembly, and translation regulation. Remarkable advancements have been made in elucidating the molecular interactions between m^5C writers, demethylases, readers, and RNAs. Among these, DNA methyltransferases *(DNMTs)* and NOP2/Sun domain family members (*NSUNs*) function as m^5C writers, catalyzing the methylation of mRNA, while ten-eleven translocation proteins (*TETs*) serve as m^5C demethylases, primarily responsible for removing m^5C modifications from mRNA. Additionally, proteins such as alpha-ketoglutarate-dependent dioxygenase ALKB homolog 1 (ALKBH1), RNA-binding protein ALY/REF export factor (ALYREF), and Y-Box binding protein 1 (YBX1) are capable of recognizing m5C modifications, acting as m^5C-binding proteins.[2] Notably, m^5C RNA methylation has emerged as a significant player in the progression of various cancers, spanning a range of tumor types, including breast cancer,[3] glioma,[4] prostate cancer, and neuroblastoma.[5,6] Given the positive association between m^5C and cancer migration and metastasis, targeting m^5C for cancer therapy has emerged as a promising avenue of research.

NSUN6 functions as an mRNA methyltransferase, specifically catalyzing the methylation of 5-methylcytidine (m^5C). Its role in mRNA methyltransferase activity and translation termination within the context of m^5C modification is crucial. The activity of mRNA and translation processes is enhanced upon *NSUN6* engagement with m^5C-containing mRNAs. Previous research has indicated low expression of *NSUN6* in human tumors, suggesting that high *NSUN6* expression may serve as an indicator of better outcomes in specific tumors.[7] In a study by Yang *et al*.,[8] the association between pancreatic cancer and m^5C-related genes was analyzed using quantitative polymerase chain reaction (PCR) and immunohistochemistry. Notably, *NSUN6* demonstrated promising performance in evaluating patient survival and tumor recurrence in pancreatic cancer cases.[8]

To identify novel susceptibility factors associated with m^5C modification—with a specific focus on single-nucleotide polymorphisms (SNPs) of the key m^5C modification-related gene *NSUN6* and its neighboring gene calcium voltage-gated channel auxiliary subunit beta 2 (*CACNB2*), both linked to neuroblastoma—we conducted a comprehensive case-control study. Our investigation unveiled significant associations between *NSUN6* and its neighboring gene *CACNB2* SNPs and the risk of neuroblastoma among children in Jiangsu province.

Materials and methods

Study subjects

This study was a case-control study, conducted at the Children's Hospital of Nanjing Medical University (Nanjing, Jiangsu, China) with ethical approval (No. 202112141-1) and adherence to the Declaration of Helsinki (as revised in 2024), from 2021 to 2023. Guardians provided written informed consent. Cases (n = 402): Children with histologically confirmed neuroblastoma (2021–2023), staged via the International Neuroblastoma Staging System, with complete clinical data (age, sex, tumor origin) and no prior chemotherapy/radiotherapy or recurrent disease. Controls (n = 473): Age- and sex-matched cancer-free children undergoing routine health checks (same period), with no genetic syndromes or chronic diseases. Exclusion criteria: Incomplete clinical/genetic data, prior immunosuppressive therapy, or refusal to provide samples. Our experimental flow chart is shown in Figure 1.

SNP selection and genotyping

SNP selection

SNPs were chosen based on: (1) localization in functional regions (e.g., introns with transcription factor binding sites); (2) minor allele frequency > 5% in Chinese Han populations (1000 Genomes Project); (3) linkage disequilibrium $R^2 < 0.8$ (Haploview 4.2).

Ultimately, three SNPs met these criteria (rs3740102 A>C, rs12780826 T>A, and rs61842187 G>C). The rs3740102 A>C, rs12780826 T>A, and rs61842187 G>C are located in the intron region of the *NSUN6* gene. The presumed function of these SNPs involves binding with transcription factors.

DNA extraction & genotyping

DNA extraction: Peripheral blood genomic DNA was isolated using the TIANamp Genomic/Blood DNA Kit (TianGen, Beijing, China) per the manufacturer's protocol. TaqMan assay: Genotyping was performed via TaqMan Genotyping PCR on an Applied Biosystems 7500 Fast Real-Time PCR System. Reaction system (10 μL): 5 μL 2× Master Mix, 0.25 μL 20× SNP Assay, 2 μL DNA (50 ng/μL), 2.75 μL nuclease-free water. Cycling conditions: 95°C for 10 m; 40 cycles of 95°C for 15 s, 60°C for 1 m. We employed the TaqMan SNP genotyping method to analyze these three SNPs within both the case and control groups, with 10% of samples randomly chosen for method repetition. Secondary genotyping confirmed 100% consistency.

Expression quantitative trait locus (eQTL) analysis (the Genotype-Tissue Expression (GTEx) database)

eQTL analysis used the GTEx Portal (v9). Relevant tissues (adrenal gland, tibial nerve, whole blood) were selected (linked to neuroblastoma pathogenesis). Associations between SNPs and target gene expression were extracted, including nominal *P*-values and expression fold changes.

Statistical analysis

The cases and controls were divided into different subgroups based on their age, sex, tumor origin, and International Neuroblastoma Staging System stage. We utilized the chi-square test to analyze the discrete variables and the t-test to analyze the continuous variables like age range. The goodness-of-fit chi-square test was employed to assess the Hardy-Weinberg equilibrium in controls. Multiple logistic regression analysis was conducted to calculate the odds ratio (OR) value and 95% confidence interval (CI) value after adjusting for age and sex. All of these analyses were conducted using SAS 9.4, with a significance level set at $P < 0.05$. We investigated the effect of rs3740102, rs12780826, and rs61842187 on *NSUN6* or nearby genes by querying the GTEx online website. Furthermore,

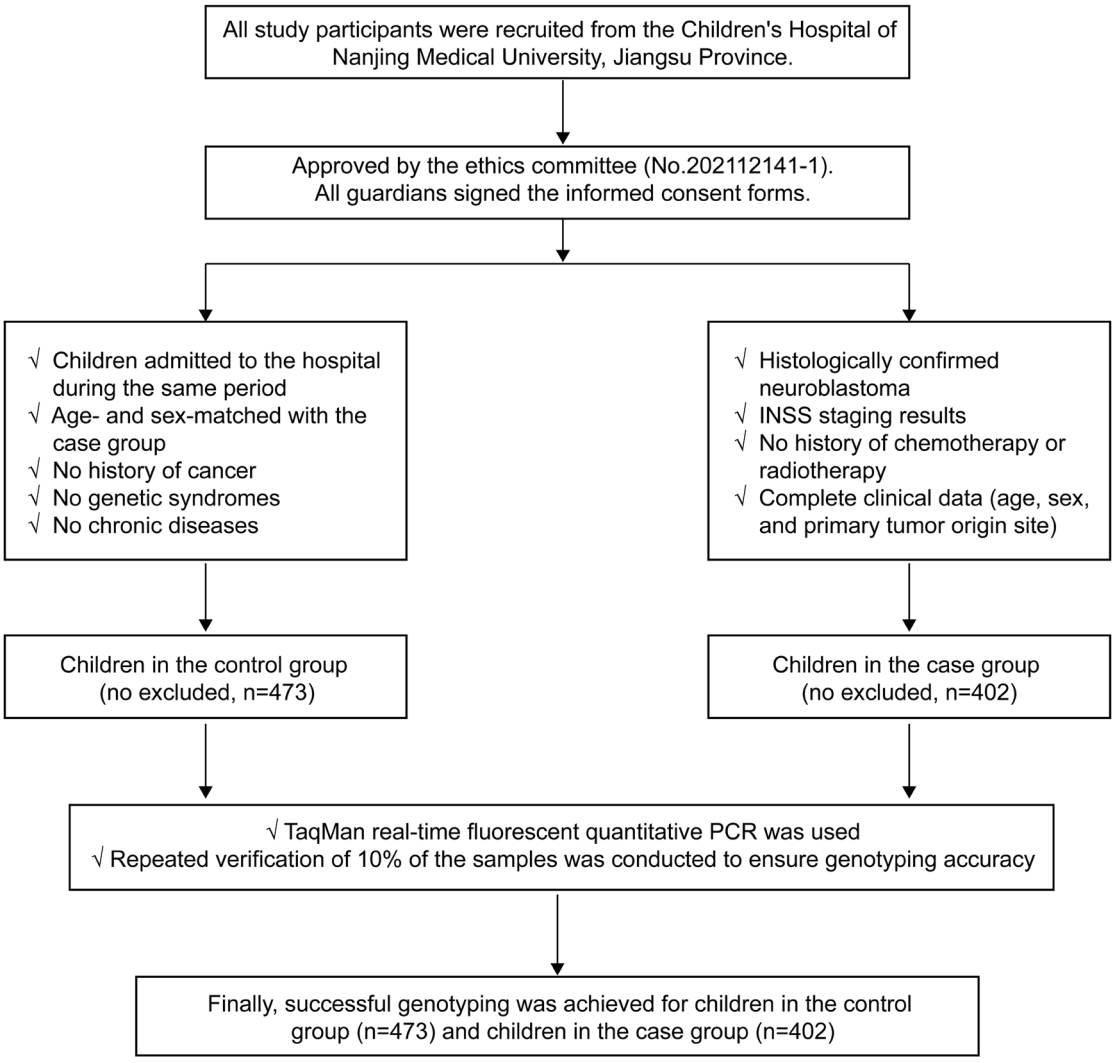

Fig. 1. Experimental flow chart. PCR, polymerase chain reaction.

the expression levels of *NSUN6* and *CACNB2* were analyzed by the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl). The relationship between *NSUN6*/*CACNB2* and overall survival (OS) and event-free survival (EFS) of neuroblastoma patients was investigated using the Sanger-box platform. Moreover, non-parametric testing was utilized to analyze differences in gene expression between two groups: those classified as high risk or without high risk of neuroblastoma, and those with or without MYCN proto-oncogene, BHLH transcription factor (*MYCN*) amplification.

Results

Association study

This study recruited 402 cases and 473 controls, all of whom were successfully genotyped. Complete demographic information for all participants is provided in Table S1. Relevant information can also be found in our previous research.[9,10] All of these gene polymorphisms (rs3740102 A>C, rs12780826 T>A, and rs61842187 G>C) conformed to Hardy-Weinberg equilibrium ($P > 0.05$) (Table 1). No significant association was found between neuroblastoma and the individual genotypes (rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC). However, compared to subjects with zero to one risk genotype, those with two to three risk genotypes were observed to have an association that may be related to lower neuroblastoma risk (2–3 vs. 0–1: adjusted OR = 0.41, 95% CI = 0.23–0.73, $P = 0.002$).

Stratified analysis

In the context of further analyzing the influence of *NSUN6* gene polymorphisms on neuroblastoma risk, we stratified our variables by age, sex, site of origin, and clinical stage for stratification (Table 2). All comparisons below refer to the contrast between two to three protective genotypes and zero to one protective genotypes. Significant risk reductions were observed in the following subgroups: ≤ 18-month subgroup (adjusted OR = 0.11, 95% CI = 0.03–0.49, $P = 0.004$), the male subgroup (adjusted OR = 0.25, 95% CI = 0.09–0.70, $P = 0.008$), the III + IV clinical stages (adjusted OR = 0.26, 95% CI = 0.14–0.49, $P < 0.0001$), the adrenal

Table 1. Association of *NSUN6* gene polymorphisms with neuroblastoma risk in children from Jiangsu province

Genotype	Cases (n = 402)	Controls (n = 473)	*P*[a]	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)[b]	*P*[b]
rs3740102 A>C (HWE = 0.559)							
AA	216 (53.73)	242 (51.16)		1.00		1.00	
AC	141 (35.07)	189 (39.96)		0.84 (0.63–1.11)	0.218	0.84 (0.63–1.11)	0.218
CC	45 (11.19)	42 (8.88)		1.20 (0.76–1.90)	0.435	1.20 (0.76–1.90)	0.436
Additive			0.955	0.99 (0.81–1.21)	0.955	0.99 (0.81–1.22)	0.955
Dominant	186 (46.27)	231 (48.84)	0.448	0.90 (0.69–1.18)	0.449	0.90 (0.69–1.18)	0.447
AA/AC	357 (88.81)	431 (91.12)		1.00		1.00	
CC	45 (11.19)	42 (8.88)	0.254	1.29 (0.83–2.02)	0.255	1.30 (0.83–2.02)	0.254
rs12780826 T>A (HWE = 0.327)							
TT	316 (78.61)	357 (75.48)		1.00		1.00	
TA	71 (17.66)	105 (22.20)		0.76 (0.55–1.07)	0.117	0.76 (0.55–1.07)	0.118
AA	15 (3.73)	11 (2.33)		1.54 (0.70–3.40)	0.285	1.54 (0.70–3.41)	0.285
Additive			0.613	0.93 (0.72–1.22)	0.614	0.93 (0.72–1.22)	0.614
Dominant	86 (21.39)	116 (24.52)	0.273	0.84 (0.61–1.15)	0.274	0.84 (0.61–1.15)	0.274
TT/TA	387 (96.27)	462 (97.67)		1.00		1.00	
AA	15 (3.73)	11 (2.33)	0.222	1.63 (0.74–3.59)	0.226	1.63 (0.74–3.60)	0.226
rs61842187 G>C (HWE = 0.157)							
GG	225 (55.97)	257 (54.33)		1.00		1.00	
GC	153 (38.06)	175 (37.00)		1.00 (0.75–1.32)	0.992	1.00 (0.75–1.32)	0.988
CC	24 (5.97)	41 (8.67)		0.67 (0.39–1.14)	0.140	0.67 (0.39–1.14)	0.139
Additive			0.312	0.90 (0.73–1.11)	0.312	0.90 (0.72–1.11)	0.309
Dominant	177 (44.03)	216 (45.67)	0.628	0.94 (0.72–1.22)	0.628	0.94 (0.72–1.22)	0.626
GG/GC	378 (94.03)	432 (91.33)		1.00		1.00	
CC	24 (5.97)	41 (8.67)	0.129	0.67 (0.40–1.13)	0.132	0.67 (0.40–1.13)	0.131
Combine protective genotypes[c]							
0–1	37 (9.20)	19 (4.02)		1.00		1.00	
2–3	365 (90.80)	454 (95.98)	0.002	0.41 (0.23–0.73)	0.002	0.41 (0.23–0.73)	0.002

[a]χ^2 test for genotype distributions between neuroblastoma patients and cancer-free controls. [b]Adjusted for age and sex. [c]Protective genotypes were carriers with rs3740102 AA/AC, rs12780826 TT/TA and rs61842187 CC genotypes. CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio.

gland origin subgroup (adjusted OR = 0.39, 95% CI = 0.17–0.90, $P = 0.027$), the retroperitoneal subgroup (adjusted OR = 0.39, 95% CI = 0.20–0.79, $P = 0.008$), and others (adjusted OR = 0.20, 95% CI = 0.05–0.77, $P = 0.019$).

Functional effects of rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes on nearby genes

Given the significant impact of these protective genotypes on neuroblastoma risk, we conducted eQTL analyses using the GTEx database for the variants rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC (Fig. 2). The rs3740102 C>A variant demonstrates an upregulation of the *NSUN6* gene expression in cultured fibroblast cells (Fig. 2a), adrenal gland (Fig. 2b), and whole blood (Fig. 2c). Additionally, the A allele of rs3740102 is associated with enhanced expression of the *CACNB2* gene in whole blood (Fig. 2d), as well as increased expression of genes such as *RP11-499P20.2* (Fig. 2e and f) and *ARL5B* (Fig. 2g and h). The rs61842187 G>C variant upregulates *NSUN6* gene expression in cultured fibroblast cells (Fig. 2i), while downregulating *ARL5B* gene expression in whole blood (Fig. 2j). Furthermore, the rs61842187 G>C variant is correlated with decreased expression of *RP11-499P20.2* (Fig. 2k) and enhanced expression of the *CACNB2* gene in the tibial artery (Fig. 2l). The rs12780826 A allele is significantly associated with increased expression of the *NSUN6* gene in cultured fibroblast cells (Fig. 2m), and elevated expression of the *CACNB2* gene in the tibial artery (Fig. 2n). Conversely, the rs12780826 A allele is linked to decreased expression of the *ARL5B* (Fig. 2o) and *RP11-499P20.2* genes in whole blood (Fig. 2p).

Functional annotation of NSUN6 and CACNB2 genes in neuroblastoma

Since the polymorphisms rs3740102 C>A, rs12780826 T>A, and

Table 2. Stratification analysis for the association between *NSUN6* genotypes and neuroblastoma susceptibility in Jiangsu children

Variables	rs61842187 (cases/controls)		Adjusted OR[a] (95% CI)	*P*[a]	Protective genotypes (cases/controls)		Adjusted OR[a] (95% CI)	*P*[a]
	GG/GC	CC			0–1	2–3		
Age, month								
≤18	134/127	5/12	0.40 (0.14–1.15)	0.090	16/2	123/137	0.11 (0.03–0.49)	0.004
>18	244/305	19/29	0.82 (0.45–1.50)	0.515	21/17	242/317	0.62 (0.32–1.20)	0.154
Gender								
Females	178/205	13/20	0.75 (0.36–1.55)	0.436	21/14	170/211	0.54 (0.27–1.09)	0.084
Males	200/227	11/21	0.59 (0.28–1.26)	0.176	16/5	195/243	0.25 (0.09–0.70)	0.008
Sites of origin								
Adrenal gland	87/432	6/41	0.72 (0.30–1.75)	0.467	9/19	84/454	0.39 (0.17–0.90)	0.027
Retroperitoneal	154/432	13/41	0.89 (0.46–1.71)	0.726	16/19	151/454	0.39 (0.20–0.79)	0.008
Mediastinum	115/432	5/41	0.46 (0.18–1.19)	0.108	7/19	113/454	0.67 (0.27–1.64)	0.378
Others	18/432	0/41	/	/	3/19	15/454	0.20 (0.05–0.77)	0.019
Clinical stages								
I + II + 4s	164/432	9/41	0.57 (0.27–1.20)	0.137	9/19	164/454	0.83 (0.37–1.89)	0.660
III + IV	149/432	14/41	0.99 (0.52–1.87)	0.969	22/19	141/454	0.26 (0.14–0.49)	<0.0001

[a]Adjusted for age and gender, omitting the correspondence factor. CI, confidence interval; OR, odds ratio.

rs61842187 G>C have significant effects on *NSUN6* mRNA expression, we further investigated the relationship between *NSUN6* mRNA levels and neuroblastoma prognosis using the R2: Genomics Analysis and Visualization Platform. Kaplan–Meier survival analysis was employed to predict the OS and EFS in neuroblastoma cases from the GSE62564 dataset (Fig. 3). Our analysis revealed that higher *NSUN6* mRNA expression (n = 249) is associated with better OS (n = 249; *P* = 2.54e-03; Fig. 3a) and EFS (n = 249; *P* = 7.90e-04; Fig. 3b) compared to neuroblastoma patients with lower *NSUN6* mRNA expression. Similarly, elevated *CACNB2* mRNA expression is associated with better prognosis, as evidenced by better OS (n = 249; *P* = 6.35e-06; Fig. 3c) and EFS (n = 249; *P* = 4.64e-06; Fig. 3d). Box plots were used to illustrate differences in *NSUN6* and *CACNB2* expression between high-risk and low-risk groups, as well as between patients with and without *MYCN* amplification. The results indicated that increased *NSUN6* expression is associated with a potential lower risk of neuroblastoma (Fig. 4a) and the absence of *MYCN* amplification (Fig. 4b). Likewise, a higher level of *CACNB2* is associated with a potential lower risk of neuroblastoma (Fig. 4c) and the absence of *MYCN* amplification (Fig. 4d).

Discussion

It is challenging to devise a universal therapy for high-risk neuroblastoma patients due to the specific heterogeneity of their biological, morphological, clinical, and genetic characteristics.[11] With advancements in understanding the genomic aberrations and disrupted pathways associated with neuroblastoma, genome-wide association studies, high-throughput genome analysis, transcriptional profiling, and genome sequencing offer new therapeutic targets to enhance patient survival rates. Novel molecular treatments may be developed to target these genomic aberrations and disrupted pathways directly.[1] However, conducting genome-wide association studies using Bonferroni correction may result in underpowered heritability estimates.[12] In our previous studies, we elucidated the relationship between neuroblastoma and m^5C methyltransferase genes, including *TET2*,[10] *TET1*,[9] *ALKBH1* rs2267755,[13] and *NSUN2* rs13181449.[14] As a type of m^5C enzyme, *NSUN6* exhibits strong substrate specificity, enabling it to install m^5C72 by targeting $tRNA^{Cys/Thr}$.[7,15] However, the biological functions and mechanisms of *NSUN6* for m^5C modification are still unknown.[16] Accordingly, we carried out this study to elucidate the correlation between neuroblastoma and the *NSUN6* gene.

Our study demonstrated that *NSUN6* rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes were associated with protective effects. Compared to individuals with zero to one protective genotype, those with a combination of two to three protective genotypes were observed to have an association that may be related to lower neuroblastoma risk. Even after adjusting for age, gender, tumor origin sites, and clinical stages, the combination of two to three *NSUN6* protective genotypes remained associated with lower neuroblastoma susceptibility. Additionally, stratified analysis showed that this association was notably more significant across all subgroups, and this result was supported by Bonferroni correction. To verify the effect of *NSUN6* rs3740102, rs12780826, and rs61842187 genotypes, we conducted eQTL analyses in the GTEx database to investigate the functional effect of rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes on nearby genes. Additionally, we utilized Kaplan-Meier survival analysis to explore the functional annotation of *NSUN6* and *CACNB2* genes in neuroblastoma. Ultimately, the results indicated that higher expression of *NSUN6* and *CACNB2* genes was associated with better prognosis, low risk, and non-*MYCN* amplification.

MYCN amplification accounts for 20–25% of neuroblastoma patients, often resulting in poor prognosis and a significant proportion of cancer-related deaths.[17] Our research found that higher expression of *NSUN6* always accompanied lower *MYCN* ampli-

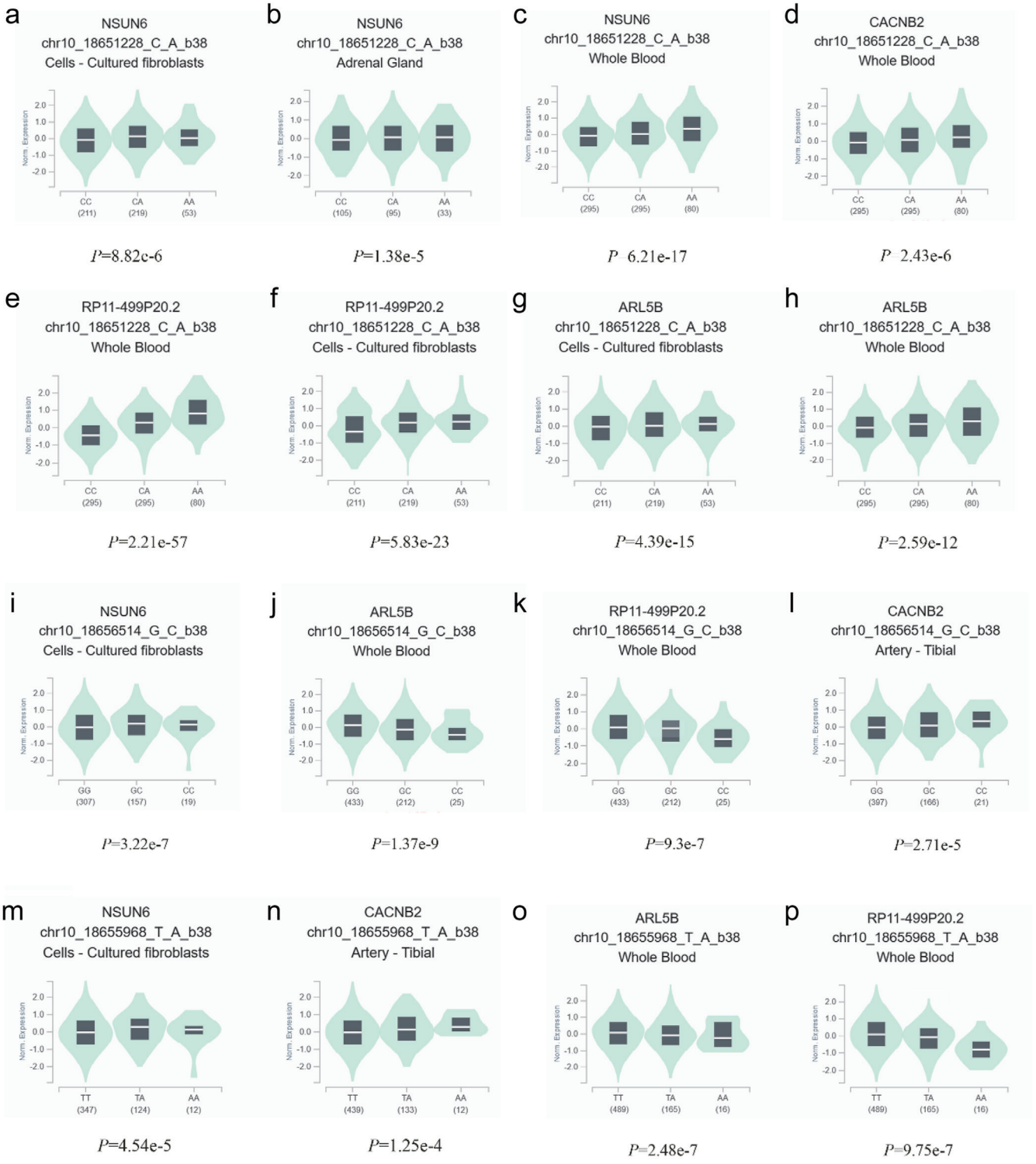

Fig. 2. eQTL analysis for rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC. The eQTL analysis reveals the functional implication of the rs3740102 C>A variant on *NSUN6* gene expression (a–c), in cultured fibroblast cells (a), adrenal gland (b), and whole blood (c). Moreover, the rs3740102 A allele significantly enhances the expression of the *CACNB2* gene (d), *RP11-499P20.2* gene (e–f), and *ARL5B* gene (g–h). Additionally, the rs61842187 G>C variant increases the expression of both *NSUN6* and *CACNB2* genes (i, l), while in whole blood, it decreases the expression of *ARL5B* (j) and *RP11-499P20.2* (k). In cultured fibroblast cells, the rs12780826 T>A variant is significantly associated with increased *NSUN6* mRNA expression (m), and in the tibial artery, it correlates with higher levels of *CACNB2* mRNA expression (n). Furthermore, in whole blood, rs12780826 T>A correlates with elevated levels of *ARL5B* (o) and *RP11-499P20.2* mRNA expression (p). eQTL, expression quantitative trait locus; mRNA, messenger RNA.

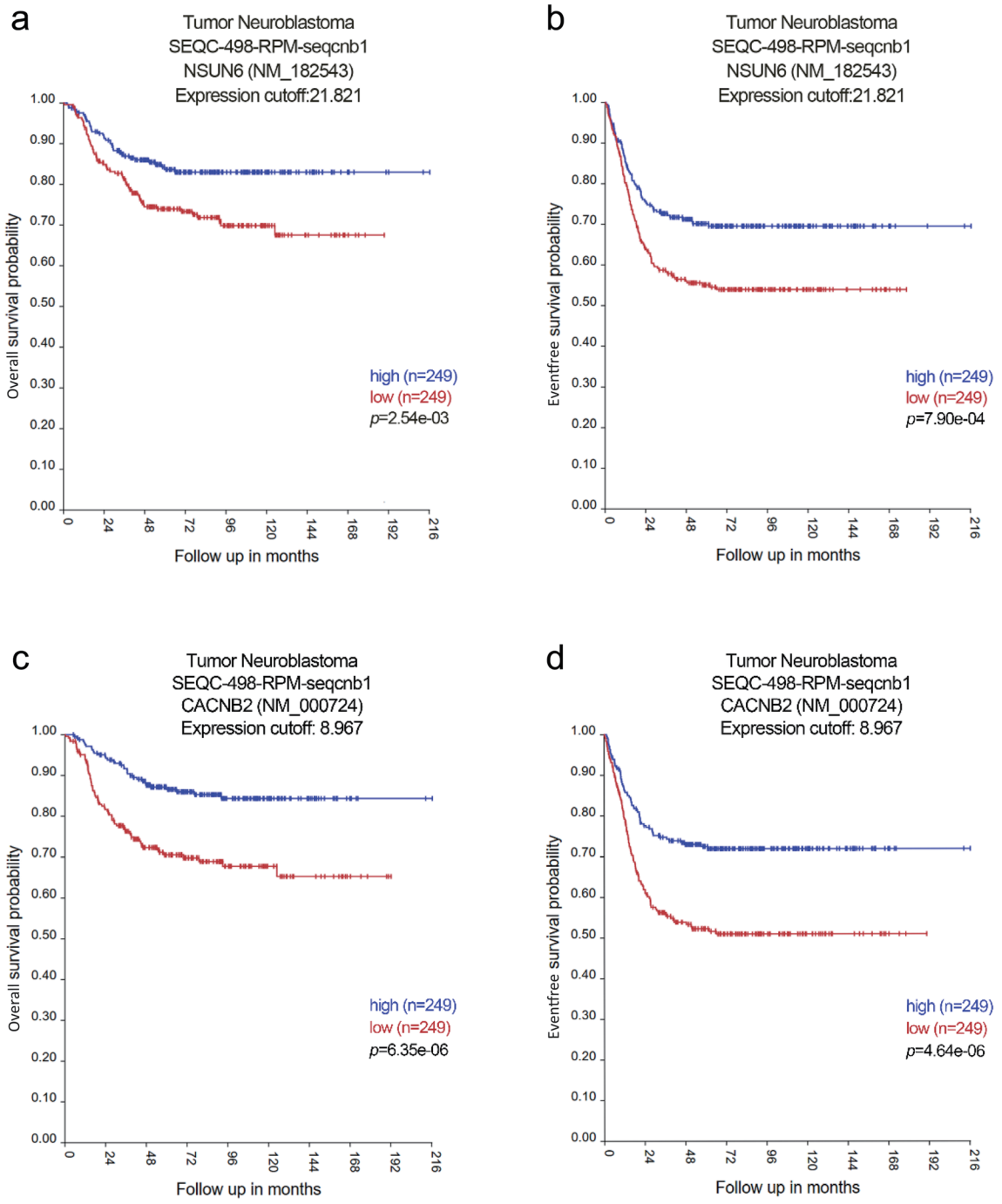

Fig. 3. Elevated *NSUN6* gene expression significantly improves overall survival (OS) probability (a) and event-free survival (EFS) probability (b) when compared to lower expression levels. Similarly, increased expression of the *CACNB2* gene is significantly associated with improved OS probability (c) and EFS probability (d).

fication, which is characteristic of low risk and good prognosis. Recently, *NSUN6* has shown survival benefits among glioblastoma patients and in other cancers.[7,8,18,19] Our study also confirmed that higher expression of the *NSUN6* gene can improve the prognosis of neuroblastoma. Selmi T and his colleagues revealed that in certain cancer types, high expression of *NSUN6* in mice indicated a better patient survival rate.[7]

In lung cancer, Lu *et al*.[20] found that NM23-H1 was expressed at low levels while *NSUN6* was overexpressed. Further testing confirmed that high levels of *NSUN6* can regulate NM23-H1 in an m^5C-dependent manner to inhibit tumor proliferation, migration, and epithelial-mesenchymal transition.[20] Temozolomide is a type of therapy for glioblastoma cancer. Awah *et al*.[18] presented evidence that *NSUN6* can influence the response to Temozolomide

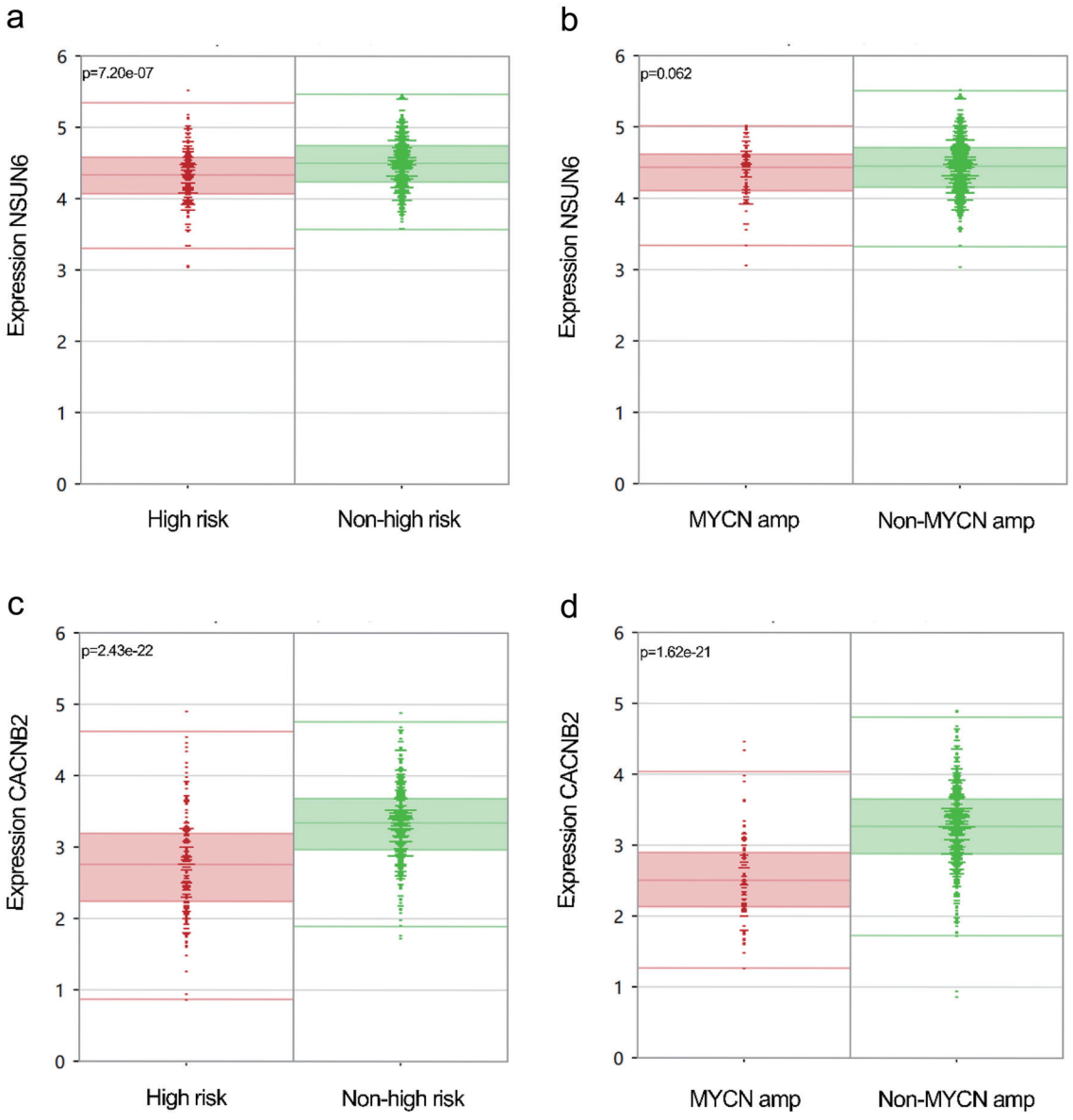

Fig. 4. Functional expression of *NSUN6* and *CACNB2* genes is observed in neuroblastoma (a–d). Higher expression of the *NSUN6* gene is correlated with a low risk of neuroblastoma (a) and absence of *MYCN* amplification (b). Similarly, elevated expression of the *CACNB2* gene is linked to neuroblastoma with low risk (c) and absence of *MYCN* amplification (d).

therapy through *NELFB* and *RPS6BK2*, which are regulated by m^5C in glioblastoma cancer. *NSUN6* rs12780826 T>A may cause the variant of intron and upstream transcript; *NSUN6* rs3740102 C>A, rs61842187 G>C may lead to variants in the 5' UTR, intron, and upstream transcript. However, there are no reports detailing the clinical consequences of these three SNPs.[21] In this study, we found that the combination of *NSUN6* rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes is associated with a potential lower neuroblastoma risk. Further analyses indicated that higher *NSUN6* gene expression is correlated with a potential lower neuroblastoma risk and the absence of *MYCN* amplification. Overall, the potential association of these genotypes with neuroblastoma prognosis may be related to the following: the A allele of rs3740102 A>C, A allele of rs12780826 T>A, and C allele of rs61842187 C>G may be linked to the potential upregulation of *NSUN6* mRNA expression and a lower likelihood of *MYCN* amplification, and this expression pattern (higher *NSUN6*, lower *MYCN* amplification) is in turn associated with better neuroblastoma prognosis. As a member of the voltage-gated superfamily characteristic of calcium channels, *CACNB2* can encode a subunit of channel protein in voltage-dependent calcium channels. Recent studies revealed that *CACNB2* plays an important role in many cancers, including breast cancer,[22] lung cancer, and gastric cancer.[23,24] Masuelli *et al*.[22] demonstrated that in left-side breast tumors, *CACNB2* can participate in a 6-ion channel-gene signature. This signature, comprising six ion channel genes, was inversely correlated with DNA methylation writers and cancer progression markers such as stemness and proliferation. Our research suggests

that *NSUN6* rs3740102 C>A, rs61842187 G>C, and rs12780826 T>A may be associated with the potential upregulation of *CACNB2* expression; this potential upregulation of *CACNB2* expression is in turn associated with a potential lower neuroblastoma risk, the absence of *MYCN* amplification, and better patient prognosis.

This is the inaugural revelation of the association between *NSUN6* and the risk of neuroblastoma, and we have also clarified the potential mechanism. Our survey encompassed a large sample from Jiangsu, China, enhancing the credibility of our study. However, there are some limitations. Firstly, our subjects were recruited only from Nanjing, China, which may introduce sampling error and reduce the credibility of extending our conclusions. Secondly, being a case-control study, further animal trials will be necessary to validate and elucidate the potential mechanism.[25]

In future studies, further functional experiments are needed to clarify the regulatory mechanisms of *NSUN6* SNPs on *CACNB2* expression and *MYCN* amplification. Multi-center, multi-ethnic population validations should be conducted to confirm the generalizability of our findings. Additionally, exploring the potential of *NSUN6*/*CACNB2*-related markers in clinical risk stratification and personalized treatment may provide new insights for neuroblastoma management.

Conclusions

Our study found *NSUN6* rs3740102 AA/AC, rs12780826 TT/TA, and rs61842187 CC genotypes can improve the prognosis of neuroblastoma by increasing the expression *NSUN6* gene, while decreasing the amplification of *MYCN*. This conclusion has implications for prognosis prediction and suggests avenues for further mechanistic studies in neuroblastoma.

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Conflict of interest

The authors have declared that they have no competing interests.

Author contributions

Conceptualization (ZZ), methodology (SJ, YS, CZ), formal analysis and investigation (SJ, YH, CZ), writing - original draft preparation (SJ), writing - review and editing (JH), funding acquisition (ZZ), resources (HW, WZ), and supervision (ZZ, CZ). All authors have approved the final version and publication of the manuscript.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2024). Approval was obtained from the institutional review board of Guangzhou Women and Children's Medical Center (Ethical Approval No: 202016601) and the Children's Hospital of Nanjing Medical University (Approval No: 202412006-1). Guardians provided written informed consent.

Data sharing statement

All the data were available upon request from the corresponding authors.

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