BMC Cancer

RESEARCH

Open Access

Association between *MYCN* gene polymorphisms and neuroblastoma susceptibility: a case-control study in Chinese children from Jiangsu Province

Jiabin Liu[1,2†], Mengzhen Zhang[2†], Yu Ouyang[1†], Jiaming Chang[2], Wenli Zhang[2], Chunlei Zhou[3], Jing He[1,2*] and Xinxin Zhang[1,2*]

Abstract

Background Neuroblastoma, developed from the sympathetic nervous system, is a deadly childhood malignancy. There is an urgent need to elucidate its intricated etiology. *MYCN* amplification leads to aggressive neuroblastoma and represents a powerful marker of poor prognosis. However, the correlation between *MYCN* gene polymorphisms and neuroblastoma susceptibility remains largely unknown in Chinese Han children.

Methods We conducted a case-control study to evaluate the associations between *MYCN* gene polymorphisms and neuroblastoma susceptibility, involving 402 cases and 473 controls from Jiangsu Province, China. The association strength between the studied polymorphisms and neuroblastoma susceptibility was quantified using odds ratios and 95% confidence intervals.

Results Four studied polymorphisms (rs57961569 G > A, rs9653226 T > C, rs13034994 A > G, and rs60226897 G > A) were significantly associated with neuroblastoma susceptibility. Stratified analysis of two polymorphisms (rs13034994 A > G and rs60226897 G > A) demonstrated stronger associations with neuroblastoma susceptibility in specific subgroups. Moreover, survival analysis demonstrated elevated *MYCN* expression in high-risk patients, with reduced expression correlating to improved survival outcomes.

Conclusion Our study indicated that *MYCN* gene polymorphisms are significantly associated with neuroblastoma susceptibility in the eastern Chinese population and that high expression of the *MYCN* gene may suggest a poor prognosis. Nevertheless, further verification should be conducted with large-scale and well-designed studies to confirm our findings.

†Jiabin Liu, Mengzhen Zhang and Yu Ouyang contributed equally to this work.

*Correspondence:
Jing He
hejing@gwcmc.org; hejing198374@gmail.com
Xinxin Zhang
zhangxinxin91@126.com

Full list of author information is available at the end of the article

Keywords *MYCN*, Polymorphism, Neuroblastoma, Susceptibility

Introduction

Neuroblastoma is the most common extracranial malignancy in childhood, with the mean age at diagnosis of approximately 17 months [1]. Its incidence ranks fourth among pediatric cancers in China and is approximately 7.7 per million [2]. Neuroblastoma comprises 8-10% of all pediatric neoplasms and contributes to 15% of cancer-associated childhood mortality [3]. This neoplasm exhibits high heterogeneity with a wide spectrum of clinical patterns, varying from spontaneous improvement without any clinical intervention or minimal chemotherapy to aggressive phenotypes and poor prognosis despite multiple intensive comprehensive therapies [4]. The survival rate of patients with neuroblastoma sharply decreases with the progression of the disease. The survival rate for early low-risk patients is over 95%, while for those in clinical stage III, it drops to only 60%, and for stage IV patients, it plummets to just 20% [5]. Advanced neuroblastoma patients typically present with aggressive and metastatic disease at the time of diagnosis, which poses a significant challenge in treating these high-risk individuals and contributes to their poor prognosis. Therefore, improving the assessment of risk factors and accurately identifying genetic markers is crucial for effectively screening high-risk populations and precisely predicting patient outcomes.

The pathology and etiology of neuroblastoma are rather complicated and remain poorly understood involving the interaction of environmental and genetic factors. Studies have shown that genetic variants of the *PHOX2B* [6] and *ALK* [7] genes are related to the risk of familial neuroblastoma. The genetic etiology of sporadic neuroblastoma is not fully known. Several environmental factors, such as hydrocarbons, wood dust, welding flux, and radiation sources, may predispose individuals to neuroblastoma [8, 9]. However, only a small fraction of individuals suffer from neuroblastoma after being exposed to the above environmental factors. Therefore, genetic factors have gradually been recognized as the main causes of the neuroblastoma. An increasing number of studies suggest that hereditary genetic polymorphisms are associated with neuroblastoma susceptibility and may predispose to neuroblastoma [10].

Single nucleotide polymorphisms (SNPs) are well-known to be involved in disease susceptibility, prognosis, and survival. SNPs are also extensively employed in the exploration of disease etiology, treatment optimization, and prognosis evaluation for various complex diseases, including neuroblastoma. Genome-wide association studies (GWASs) have been powerful tools to explore the genetic etiology of human disorders. Over the last decade, several GWASs in neuroblastoma have identified multiple independent susceptibility loci in the *CASC15* [11], *BARD1* [12], *HSD17B12*, *DUSP12*, *IL31RA*, *DDX4* [13], *LMO1* [14], *LIN28B*, *HACE1* [15], *CPZ* and *MLF1* [16]. In addition, neuroblastoma susceptibility loci in the *CDKN1B* [17], *XPG* [18], and *NEFL* [19] genes have also been found via the candidate gene method. The above findings highlight the importance of SNPs in neuroblastoma susceptibility. However, many disease-associated SNPs remain to be discovered. Therefore, extensive research is needed to further elucidate the genetic etiology of neuroblastoma.

MYCN is an important member of the *MYC* oncogene family, which was first discovered in neuroblastoma cell lines [20]. *MYCN* functions by assembling a heterodimer with *MYC*-associated protein X and then binding to the E-box regulatory motif at the promoters of target genes to regulate transcriptions [21]. *MYCN* plays key roles in numerous cytophysiological processes, including mainly cell growth [22], differentiation [23], proliferation [24], and apoptosis [25]. Its dysregulation has been reported to be involved in the initiation and progression of malignancy [26, 27]. Furthermore, *MYCN* amplification is a common and crucial genetic event in neuroblastoma, occurring in 20–25% of neuroblastoma cases and leading to aggressive behaviors and malignant progression. The amplification of *MYCN* is generally considered a reliable genetic marker of poor prognosis in neuroblastoma patients [28, 29]. However, few studies have reported associations between *MYCN* gene variants and neuroblastoma susceptibility. In this current study, four potentially functional SNPs (rs57961569 G > A, rs9653226 T > C, rs13034994 A > G, and rs60226897 G > A) in the *MYCN* gene were chose for analysis, all of them were located in the transcription factor binding sites, a key region for regulation of gene transcription. SNPs in this region may affect the binding of transcription factor then modifying gene expression. Considering the crucial roles of *MYCN* in neuroblastoma progression and the potential regulation of SNPs in the *MYCN* gene, it is biologically plausible to propose that functional SNPs in the *MYCN* gene may change its protein expression or structure. This alteration could subsequently disrupt the normal transcriptional regulation of downstream target genes, leading to cell dysfunction and eventually contributing to carcinogenesis and neuroblastoma susceptibility.

Therefore, we performed the current case-control study to assess the association between the *MYCN* gene SNPs and neuroblastoma susceptibility. These findings may help identify novel genetic markers for high-risk neuroblastoma population screening.

Materials and methods

Study subjects

In this case-control study, 402 neuroblastoma patients and 473 control subjects were enrolled from Jiangsu Province, China [30, 31]. All patients were newly clinically diagnosed and histopathologically confirmed without previous treatments. The healthy control participants were randomly selected from children visiting for routine medical examinations in the same area during the same period. Age, sex, and race were matched between the cases and controls. The exclusion criteria for the participants were described in detail in our previous publications [18]. The demographic features of the study subjects are displayed in Table **S1**. Medical informed consent documents were acquired from the included subjects or their guardians before the study. The study followed the Declaration of Helsinki guidelines and the research protocol was approved by the ethics committee of the Children's Hospital of Nanjing Medical University (Approval No: 202112141-1).

Polymorphism selection and genotyping

Four potentially functional genetic variants in the *MYCN* gene (rs13034994 A > G, rs60226897 G > A, rs57961569 G > A, and rs9653226 T > C) were selected for analysis [32]. For genotyping, the extraction and purification of genomic DNA from peripheral blood were conducted with a TIANamp Blood DNA Kit (TianGen Biotech, Beijing, China). The genotyping of the selected SNPs in the diluted DNA samples was performed via the TaqMan PCR method (Figure **S1**) [33–35]. A second analysis was conducted on 10% of the randomly selected DNA samples and 100% consistency was obtained between the two genotyping results.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) status of the SNPs was tested using a goodness-of-fit χ^2 test in the controls. The two-sided χ^2 test was applied to compare the differences in demographic characteristics and genotype frequency distributions between the case and control groups. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated via the logistic regression model to evaluate the associations between the SNPs and neuroblastoma susceptibility. The adjusted ORs and counterpart 95% CIs adjusted for age and sex were determined through unconditional multivariate logistic regression analysis. In addition, we performed stratified analysis regarding age, sex, tumor site of origin, and clinical stage. We applied SAS software (version 9.4 SAS Institute, NC, USA) to carry out the statistical analysis. The results were considered statistically significant when the corresponding *P* value was < 0.005.

Survival analysis

A public microarray dataset (GSE49710) was obtained from the NCBI Gene Expression Omnibus (GEO) database. Information on *MYCN* expression was extracted from the database, and the differences between the high-risk group and the group with no high risk were analyzed [36]. Based on this dataset, the level of *MYCN* was dichotomized into low- and high-expression subgroups according to the median. The Kaplan–Meier method with the R2 database server was used for survival analysis and statistical difference in survival between the subgroups was assessed using a log-rank test.

Results

Associations between *MYCN* gene polymorphisms and neuroblastoma susceptibility

In the present study, 402 neuroblastoma patients and 473 cancer-free controls were included and successfully genotyped to analyze the relationship between *MYCN* gene polymorphisms and neuroblastoma susceptibility. As indicated in Table 1. The genotype frequencies of rs13034994 A > G ($P = 0.772$) and rs60226897 G > A ($P = 0.664$) were in HWE among the controls, while those of the other two SNPs, rs57961569 G > A ($P = 0.036$) and rs9653226 T > C ($P = 0.028$) did not follow HWE. All four candidate SNPs were significantly associated with neuroblastoma susceptibility via single-locus analysis. More specifically, we found that carriers with the rs57961569 GA genotype had a decreased neuroblastoma susceptibility compared with children harboring the GG genotype (AOR = 0.72, 95% CI = 0.54–0.95, $P = 0.022$). The rs9653226 TC genotype was also associated with a decreased neuroblastoma susceptibility against the reference TT genotype (AOR = 0.66, 95% CI = 0.49–0.88, $P = 0.005$); however, the rs9653226 CC genotype increased the neuroblastoma susceptibility under the dominant model (AOR = 1.53, 95% CI = 1.07–2.18, $P = 0.019$). Subjects with the rs13034994 GG genotype also had a greater neuroblastoma susceptibility than those with the AA or AA/AG genotype (GG vs. AA: AOR = 1.76, 95% CI = 1.04–2.97, $P = 0.036$, GG vs. AA/AG: AOR = 1.85, 95% CI = 1.10–3.10, $P = 0.019$); and the rs60226897 GA genotype had a reduced neuroblastoma susceptibility compared with the GG genotype (AOR = 0.72, 95% CI = 0.54–0.96, $P = 0.025$). In addition, compared with children without the risk genotype, those carrying the 1–4 risk genotypes had an increased neuroblastoma susceptibility (AOR = 1.46, 95% CI = 1.11–1.92; $P = 0.006$).

Stratification analysis

To assess the protective or risk effects of significant SNPs on neuroblastoma susceptibility among various subgroups, stratification analysis was further performed

Table 1 Association between *MYCN* gene polymorphisms and neuroblastoma susceptibility 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]
rs57961569 G > A (HWE = 0.036)							
GG	179 (44.53)	181 (38.27)		1.00		1.00	
GA	170 (42.29)	240 (50.74)		**0.72 (0.54–0.95)**	**0.022**	**0.72 (0.54–0.95)**	**0.022**
AA	53 (13.18)	52 (10.99)		1.03 (0.67–1.59)	0.892	1.03 (0.67–1.59)	0.895
Additive			0.370	0.91 (0.75–1.11)	0.369	0.91 (0.75–1.11)	0.367
Dominant	223 (55.47)	292 (61.73)	0.061	0.77 (0.59–1.01)	0.061	0.77 (0.59–1.01)	0.060
GG/GA	349 (86.82)	421 (89.01)		1.00		1.00	
AA	53 (13.18)	52 (10.99)	0.321	1.23 (0.82–1.85)	0.321	1.23 (0.82–1.85)	0.321
rs9653226 T > C (HWE = 0.028)							
TT	154 (38.31)	153 (32.35)		1.00		1.00	
TC	166 (41.29)	252 (53.28)		**0.65 (0.49–0.88)**	**0.005**	**0.66 (0.49–0.88)**	**0.005**
CC	82 (20.40)	68 (14.38)		1.20 (0.81–1.77)	0.366	1.20 (0.81–1.78)	0.364
Additive			0.990	1.00 (0.83–1.21)	0.990	1.00 (0.83–1.21)	0.988
Dominant	248 (61.69)	320 (67.65)	0.066	0.77 (0.58–1.02)	0.066	0.77 (0.58–1.02)	0.066
TT/TC	320 (79.60)	405 (85.62)		1.00		1.00	
CC	82 (20.40)	68 (14.38)	0.019	**1.53 (1.07–2.17)**	**0.019**	**1.53 (1.07–2.18)**	**0.019**
rs13034994 A > G (HWE = 0.772)							
AA	241 (59.95)	282 (59.62)		1.00		1.00	
AG	122 (30.35)	165 (34.88)		0.87 (0.65–1.16)	0.328	0.87 (0.65–1.16)	0.328
GG	39 (9.70)	26 (5.50)		**1.76 (1.04–2.97)**	**0.036**	**1.76 (1.04–2.97)**	**0.036**
Additive			0.366	1.10 (0.89–1.36)	0.365	1.10 (0.89–1.36)	0.365
Dominant	161 (40.05)	191 (40.38)	0.921	0.99 (0.75–1.29)	0.921	0.99 (0.75–1.29)	0.921
AA/AG	363 (90.30)	447 (94.50)		1.00		1.00	
GG	39 (9.70)	26 (5.50)	0.018	**1.85 (1.10–3.09)**	**0.020**	**1.85 (1.10–3.10)**	**0.019**
rs60226897 G > A (HWE = 0.664)							
GG	188 (46.77)	190 (40.17)		1.00		1.00	
GA	159 (39.55)	223 (47.15)		**0.72 (0.54–0.96)**	**0.025**	**0.72 (0.54–0.96)**	**0.025**
AA	55 (13.68)	60 (12.68)		0.93 (0.61–1.41)	0.720	0.93 (0.61–1.41)	0.717
Additive			0.230	0.89 (0.73–1.08)	0.230	0.89 (0.73–1.08)	0.229
Dominant	214 (53.23)	283 (59.83)	0.050	0.76 (0.58-1.00)	0.050	**0.76 (0.58–0.999)**	**0.049**
GG/GA	347 (86.32)	413 (87.32)		1.00		1.00	
AA	55 (13.68)	60 (12.68)	0.664	1.09 (0.74–1.62)	0.663	1.09 (0.74–1.62)	0.664
Combine risk genotypes [c]							
0	146 (36.32)	215 (45.45)		1.00		1.00	
1–4	256 (63.68)	258 (54.55)	0.006	**1.46 (1.11–1.92)**	**0.006**	**1.46 (1.11–1.92)**	**0.006**

OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium

[a] χ^2 test for genotype distributions between neuroblastoma patients and cancer-free controls

[b] Adjusted for age and gender

[c] Risk genotypes were carriers with rs57961569 AA, rs9653226 CC, rs13034994 GG and rs60226897 GG

for rs13034994 A > G and rs60226897 G > A by age, sex, site of tumor origin, and clinical stage. As shown in Table 2, the risk effect of rs13034994 A > G and the protective effect of rs60226897 G > A were more evident in different subgroups. Specifically, the association of the rs13034994 GG genotype with increased neuroblastoma susceptibility was more obvious among the following subgroups: age > 18 months (AOR = 2.31, 95% CI = 1.24–4.30, *P* = 0.008), girls (AOR = 2.55, 95% CI = 1.24–5.26, *P* = 0.011), neuroblastoma of mediastinum origin (AOR = 2.10, 95% CI = 1.04–4.22, *P* = 0.038), and advanced stage (AOR = 2.42, 95% CI = 1.31–4.47, *P* = 0.005). Compared with the GG genotype, the rs60226897 GA/AA genotype significantly decreased neuroblastoma susceptibility in the following subgroups: males (AOR = 0.64, 95% CI = 0.44–0.93, *P* = 0.020), retroperitoneal origin (AOR = 0.65, 95% CI = 0.46–0.93, *P* = 0.017), and late-stage disease (AOR = 0.64, 95% CI = 0.45–0.91, *P* = 0.014). In addition, the combined analysis revealed that carriers harboring 1–4 risk genotypes had significantly greater neuroblastoma susceptibility than those with no risk genotype in subgroups aged > 18 months (AOR = 1.44, 95% CI = 1.03-2.00, *P* = 0.032), males (AOR = 1.51, 95% CI = 1.04–2.20, *P* = 0.032), retroperitoneal origin

Table 2 Stratification analysis of risk genotypes with neuroblastoma susceptibility

Variables	rs13034994 (cases/controls)		AOR (95% CI)	*P*[a]	rs60226897 (cases/controls)		AOR (95% CI)	*P*[a]	Risk genotypes		AOR (95% CI)	*P*[a]
	AA/AG	GG			GG	GA/AA			0	1–4		
Age, month												
≤ 18	129/130	10/9	1.13 (0.44–2.89)	0.800	71/58	68/81	0.69 (0.43–1.10)	0.119	50/64	89/75	1.52 (0.94–2.46)	0.089
> 18	234/317	29/17	**2.31 (1.24–4.30)**	**0.008**	117/132	146/202	0.82 (0.59–1.13)	0.223	96/151	167/183	**1.44 (1.03-2.00)**	**0.032**
Gender												
Females	167/213	24/12	**2.55 (1.24–5.26)**	**0.011**	87/98	104/127	0.92 (0.63–1.36)	0.686	70/101	121/124	1.41 (0.95–2.09)	0.089
Males	196/234	15/14	1.28 (0.60–2.72)	0.529	101/92	110/156	**0.64 (0.44–0.93)**	**0.020**	76/114	135/134	**1.51 (1.04–2.20)**	**0.032**
Sites of origin												
Adrenal gland	87/447	6/26	1.19 (0.48–2.98)	0.712	37/190	56/283	1.01 (0.64–1.60)	0.958	37/215	56/258	1.25 (0.80–1.97)	0.329
Retroperitoneal	151/447	16/26	1.82 (0.95–3.48)	0.072	85/190	82/283	**0.65 (0.46–0.93)**	**0.017**	57/215	110/258	**1.61 (1.11–2.32)**	**0.011**
Mediastinum	107/447	13/26	**2.10 (1.04–4.22)**	**0.038**	53/190	67/283	0.85 (0.57–1.27)	0.421	46/215	74/258	1.34 (0.89–2.02)	0.161
Others	15/447	3/26	3.50 (0.95–12.91)	0.060	11/190	7/283	0.43 (0.16–1.13)	0.086	6/215	12/258	1.69 (0.62–4.57)	0.305
Clinical stages												
I + II + 4 s	165/447	8/26	0.84 (0.37–1.89)	0.669	72/190	101/283	0.94 (0.66–1.34)	0.726	76/215	97/258	1.06 (0.75–1.51)	0.749
III + IV	143/447	20/26	**2.42 (1.31–4.47)**	**0.005**	83/190	80/283	**0.64 (0.45–0.91)**	**0.014**	48/215	115/258	**2.00 (1.36–2.93)**	**0.0004**

AOR, adjusted odds ratio; CI, confidence interval

[a] Adjusted for age and gender

(AOR = 1.61, 95% CI = 1.11–2.32, $P = 0.011$), and stages III + IV disease (AOR = 2.00, 95% CI = 1.36–2.93, $P = 0.0004$).

Expression and prognostic analysis

Expression analysis revealed that *MYCN* was more highly expressed in high-risk neuroblastoma patients than in no-high-risk patients, and patients with no high risk had lower expression of *MYCN* (Fig. 1). Prognostic analysis revealed that high *MYCN* expression was associated with decreased overall survival (OS) (Fig. 2) and event-free survival (EFS) (Fig. 3) of neuroblastoma patients. However, the association with EFS was only marginally significant ($P = 0.060$). These results indicate a carcinogenic effect of *MYCN* on neuroblastoma.

Discussion

SNP is the most common type of genetic variant among individuals, referring to congenital mutations with a frequency of ≥ 1% in the population. The research results of the Human Genome Project showed that the genome differences among human individuals were only 0.1%, and SNPs is the main form of these differences. Researches showed that it is these numerous SNP sites that combine together to determine the differences in traits amongst individuals, including differences in disease susceptibility and drug sensitivity. Therefore, SNP has been widely included in the research scope of disease etiology exploration, disease diagnosis and treatment research, and disease prognosis evaluation, etc. Considering the pivotal roles of *MYCN* in neuroblastoma progression and the regulatory potential of SNPs, we conducted this case-control study to evaluate the relationship between potential functional *MYCN* gene polymorphisms and neuroblastoma susceptibility. In this study, we identified four *MYCN* gene susceptibility loci (rs57961569, rs9653226, rs13034994, and rs60226897) that significantly modify neuroblastoma susceptibility. Our study contributes to the understanding of the genetic predisposition of neuroblastoma patients, and further analysis of multiple loci may improve the early diagnosis and screening of high-risk populations.

The *MYCN* gene is mapped to chromosome 2p24.3 and encodes a transcriptional nuclear phosphoprotein of the *MYC* family. It is crucial in processes associated with neoplasm initiation and progression, such as proliferation, differentiation, and survival [37]. Many studies have revealed abnormal expression of the *MYCN* gene in various tumors, such as astrocytoma, small cell lung carcinomas, Wilms' tumor, and neuroblastoma [38]. Moreover, numerous studies have shown that *MYCN* amplification commonly occurs in neuroblastoma and is closely associated with aggressive phenotypes and poor survival. *MYCN* amplification is the most

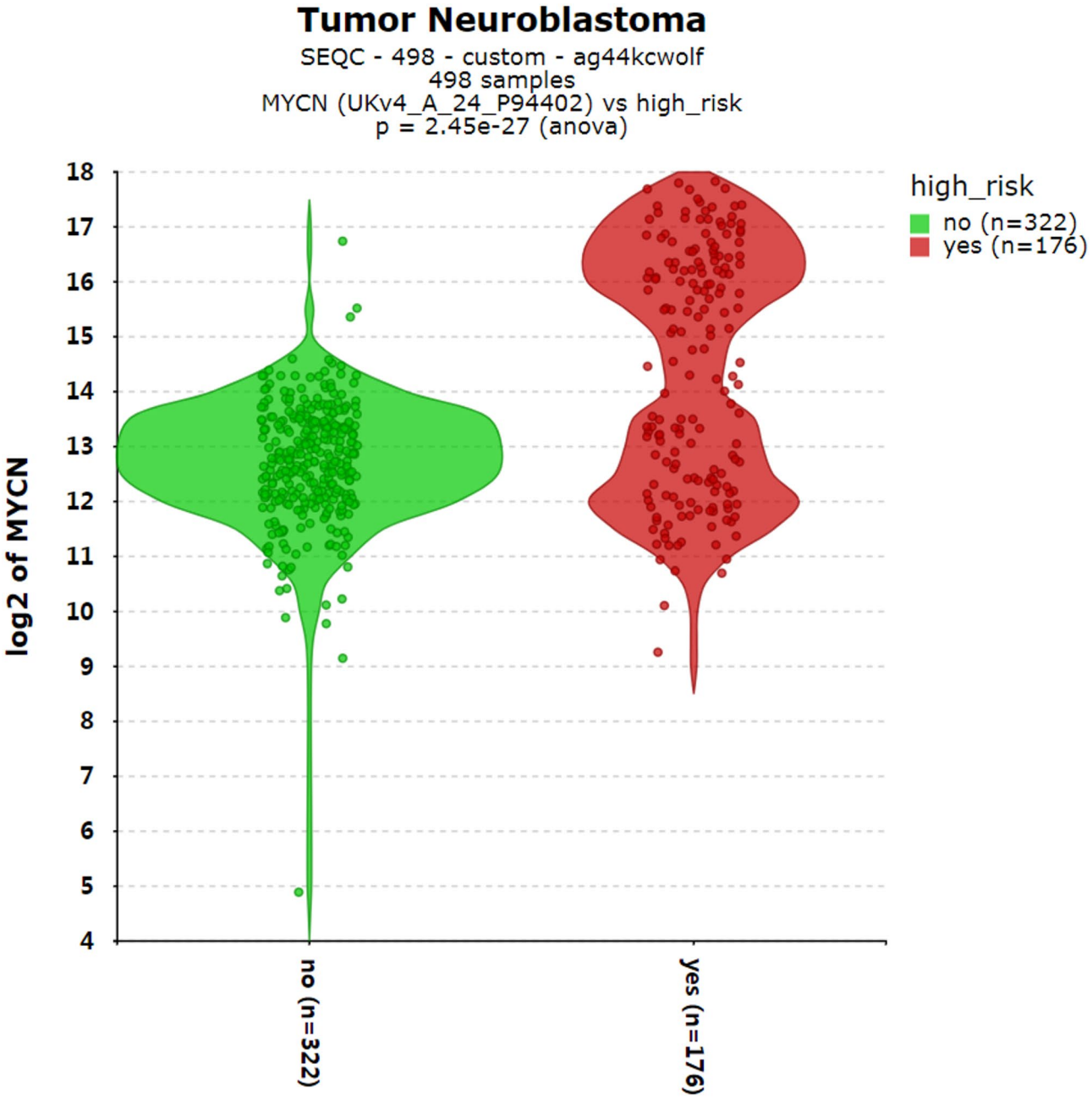

Fig. 1 Expression analysis of *MYCN* in neuroblastoma patients at high risk and not at high risk from a public GEO dataset

important molecular marker indicating advanced-stage disease and poor outcomes in neuroblastoma patients [39–42]. One study conducted by Tanaka et al. demonstrated that *MYCN* amplification enhances the migration and invasion of neuroblastoma cells by downregulating integrin α1 [43]. Rouah et al. reported that a decreased level of *MYCN* was related to neuron terminal differentiation [44]. Janardhanan et al. reported that silencing *MYCN* induced the differentiation and apoptosis of neuroblastoma cells [45]. Zaatiti et al. also revealed that the knockout of the *MYCN* gene reduced the migration and proliferation of a neuroblastoma cell line [46]. In addition, *MYCN* is involved in immune evasion by dampening the expression of ligands on the neuroblastoma cell surface, which bind to NK-cell-activating receptors for NK cell activation [47]. These studies revealed the important roles of *MYCN* in neuroblastoma progression; however, few studies have investigated the associations between *MYCN* genetic variants and neuroblastoma susceptibility. One study involving 243 cases and 247 controls performed by Dahlin et al. revealed no significant association between *MYCN* rs922 G > A variant and

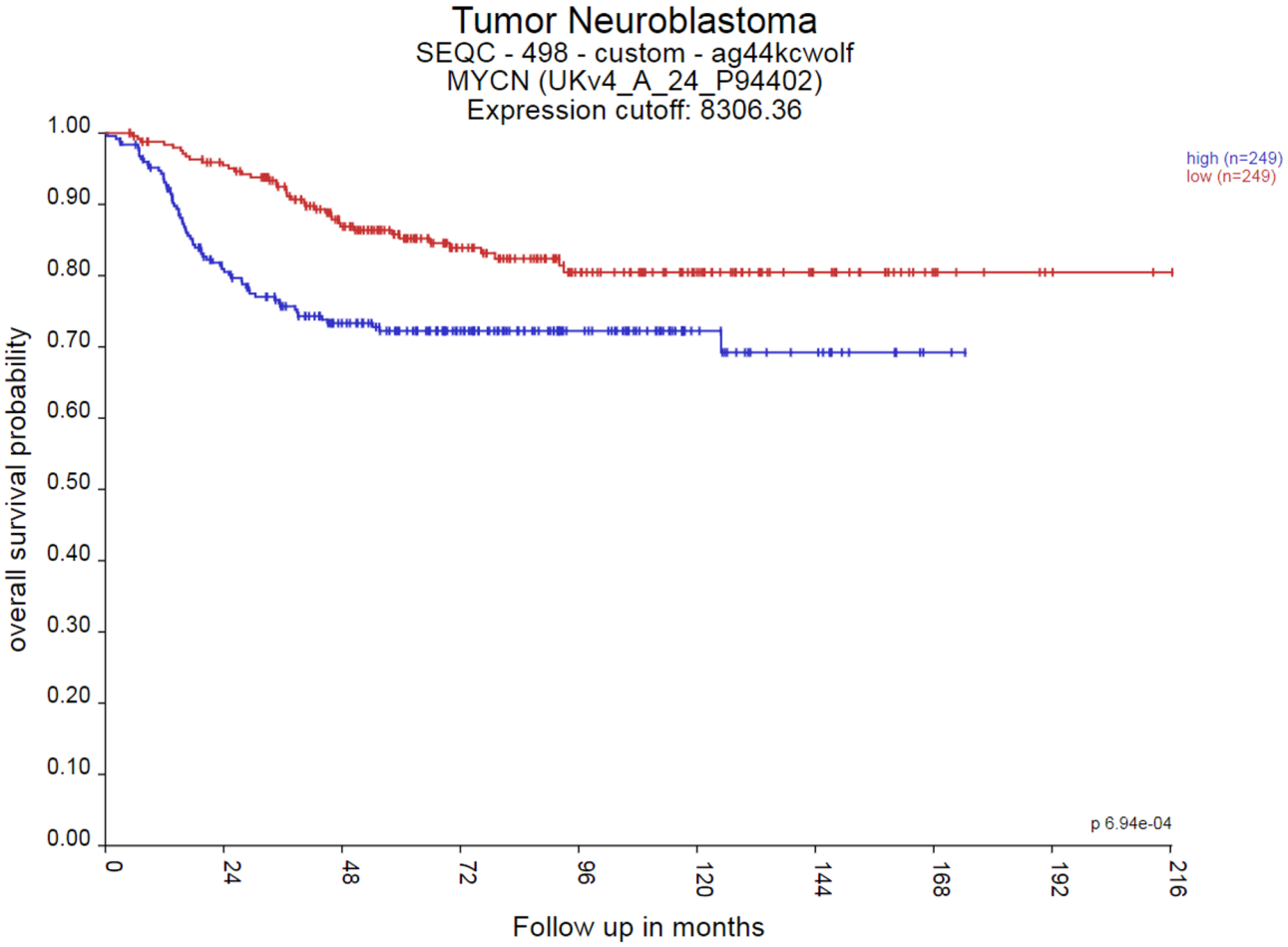

Fig. 2 Prognostic analysis between the expression of *MYCN* and the overall survival probability of neuroblastoma patients

medulloblastoma susceptibility in Swedish and Danish individuals, which was the first study to explore the association between *MYCN* gene polymorphisms and cancer susceptibility [48]. Our recent study also revealed no significant relationship between *MYCN* polymorphisms and Wilms tumor risk in Chinese children [49]. However, our other research revealed that one of the *MYCN* genetic variants, rs57961569 G > A, was significantly associated with neuroblastoma susceptibility in a three-center case-control study, whereas other variants (rs9653226 T > C, rs13034994 A > G, and rs60226897 G > A) were not [32].

Herein, we carried out a case-control study to assess the associations between four SNPs of the *MYCN* gene and neuroblastoma susceptibility in a population from Jiangsu Province, China. All the studied SNPs were located at the promoter region of the *MYCN* gene. Our results revealed that all four selected SNPs were significantly associated with neuroblastoma susceptibility. Specifically, we found that subjects with the rs57961569 GA, rs9653226 TC, or rs60226897 GA genotypes had a lower neuroblastoma susceptibility than those with the reference genotype and that carriers with the rs13034994 GG genotype had a greater neuroblastoma susceptibility than those with the AA genotype. More prominent associations were observed among the different subgroups in the stratification analysis. The effects of *MYCN* gene polymorphisms on neuroblastoma susceptibility may be age-, sex-, site- and origin-, and clinical stage dependent. These contradictory results may result from differences in the study population, tumor type, or other factors. It is possible that these significant SNPs maybe just a chance discovery owing to the relatively small sample size. Well-designed research with larger samples should be conducted to confirm our findings.

Moreover, the expression analysis revealed that *MYCN* was expressed at higher levels in the high-risk cohort than in the low-risk cohort. Survival analysis revealed better OS and EFS in neuroblastoma patients with low *MYCN* expression than in those with high *MYCN* expression. Although not statistically significant for EFS, these findings suggest a protumor role of *MYCN* in neuroblastoma progression. The candidate SNPs (especially those located at the promoter region of *MYCN*) may modify the expression of the *MYCN* gene, therefore affecting

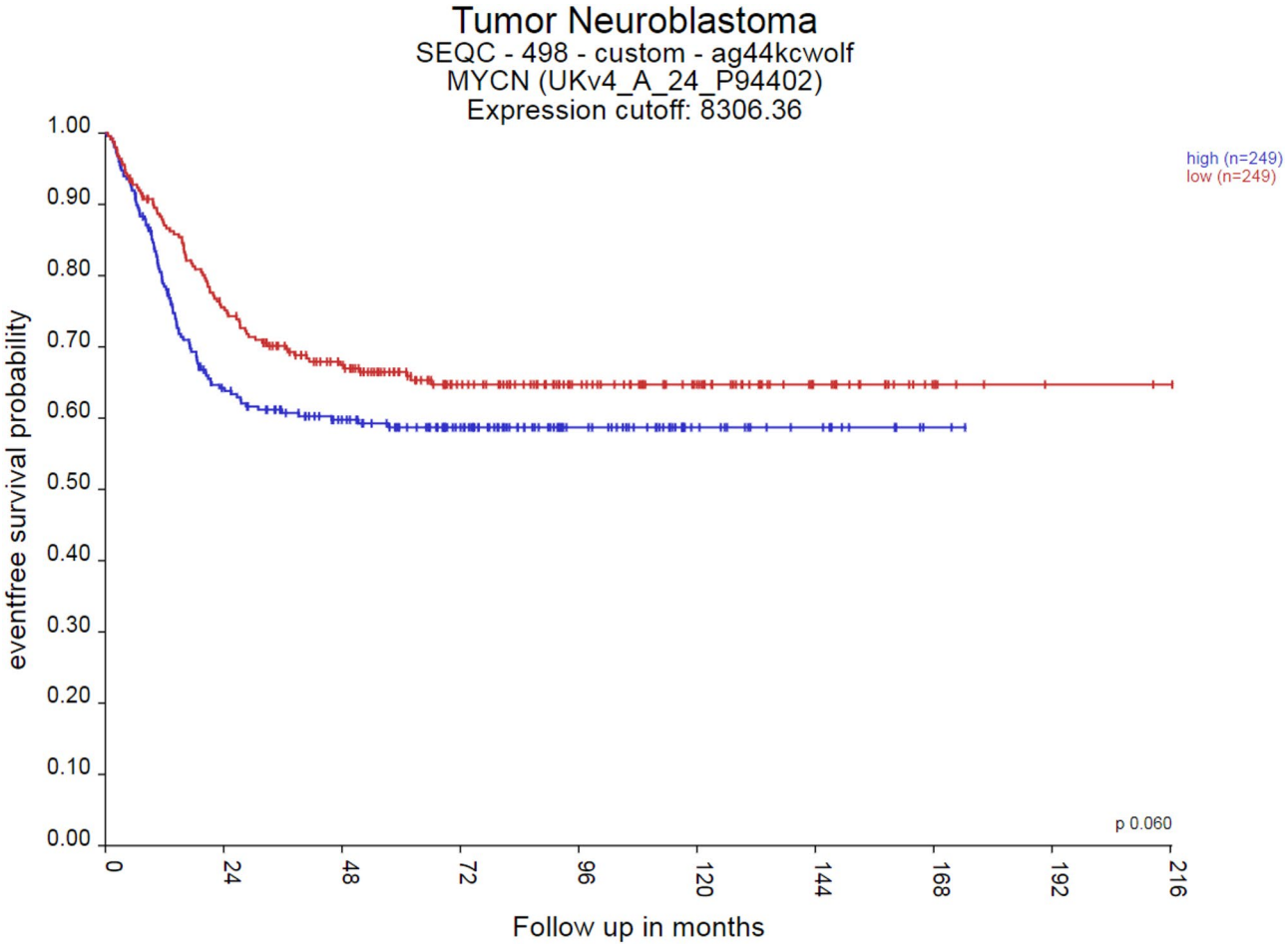

Fig. 3 Prognostic analysis between the expression of *MYCN* and the event-free survival probability of neuroblastoma patients

neuroblastoma susceptibility. This may also be one of the potential mechanisms by which associated SNPs modify neuroblastoma susceptibility. However, in-depth mechanistic studies on how significant SNPs affect the expression of *MYCN* and modify neuroblastoma susceptibility are needed in the future.

Several shortcomings should be noted in the present study. First, the sample size was small, especially in the stratification analysis, which inevitably led to decreased statistical power. Second, other potentially effective SNPs located at the *MYCN* gene should be assessed. Third, this case-control study was a single-center study, and the subjects included in the research were of Chinese origin; hence, the conclusions of this study may not be applicable to other regions and ethnicities. Fourth, environmental factors should be evaluated, as the complicated etiology of neuroblastoma involves interactions between multiple environmental and genetic factors. Fifth, mechanistic studies should be conducted to elucidate the underlying mechanisms by which *MYCN* genetic polymorphisms affect neuroblastoma susceptibility. Sixth, the unavailableness of complete sample information, such as environmental exposure and *MYCN* status of the investigate subjects.

Conclusion

In summary, our current study suggests that genetic variants of the *MYCN* gene could significantly modify neuroblastoma susceptibility in eastern China. These findings support the exploitation of new diagnostic genetic markers of neuroblastoma from *MYCN* genetic variants. Nevertheless, our findings should be confirmed by well-designed and multi-center studies involving larger samples. Moreover, future studies should elucidate the mechanisms through which *MYCN* variants contribute to neuroblastoma pathogenesis.

Abbreviations

SNP	Single nucleotide polymorphism
GWAS	Genome-wide association study
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
CI	Confidence interval
GEO	Gene expression omnibus
OS	Overall survival
EFS	Event-free survival

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14310-w.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
None.

Author contributions
All the authors contributed significantly to this work. C.Z. collected the samples and data; J.L., M.Z., Y.O., J.C., W.Z., X.Z. and J.H. performed the research study; W.Z. and J.H. analyzed the data and prepared all the figures and tables; J.H. and X.Z. designed the research study; and J. L., M.Z., Y.O. and J.H. wrote the paper. All authors have read and approved the final manuscript to be published.

Funding
This study was supported by grants from the National Natural Science Foundation of China (No: 32300473), Guangdong Basic and Applied Basic Research Foundation (No: 2023A1515220053), and Guangzhou Science and Technology Project (No: 202201020622).

Data availability
The original contributions presented in the study are provided in the article/Supplementary Materials; all the data are available upon request from the correspondence authors (Jing He or Xinxin Zhang).

Declarations

Ethics approval and consent to participate
The study followed the Declaration of Helsinki guidelines. All participants' guardians have signed informed written consent. Before conducting the research, the study obtained approval from the Children's Hospital of Nanjing Medical University's Institutional Review Committee (approval number No: 202112141-1).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Pediatric Surgery, Liuzhou Key Laboratory of Birth Defect Prevention and Control, Guangzhou Women and Children's Medical Center Liuzhou Hospital, Liuzhou 545616, Guangxi, China
[2]Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, Guangdong, China
[3]Department of Pathology, Children's Hospital of Nanjing Medical University, Nanjing 210008, Jiangsu, China

Received: 6 February 2025 / Accepted: 12 May 2025
Published online: 19 May 2025

References

1. Cheung NK, Dyer MA. Neuroblastoma: developmental biology, cancer genomics and immunotherapy. Nat Rev Cancer. 2013;13:397–411.
2. Bao PP, Li K, Wu CX, Huang ZZ, Wang CF, Xiang YM, Peng P, Gong YM, Xiao XM, Zheng Y. [Recent incidences and trends of childhood malignant solid tumors in Shanghai, 2002–2010]. Zhonghua Er Ke Za Zhi. 2013;51:288–94.
3. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL. Diller L and Weiss WA. Neuroblastoma. Nat Rev Dis Primers. 2016;2:16078.
4. Tolbert VP, Coggins GE, Maris JM. Genetic susceptibility to neuroblastoma. Curr Opin Genet Dev. 2017;42:81–90.
5. Liu J, Deng C, Lin H, Zhang X, Zhu J, Zhou C, Wu H, He J. Genetic variants of m7G modification genes influence neuroblastoma susceptibility. Heliyon. 2024;10:e23658.
6. Bourdeaut F, Trochet D, Janoueix-Lerosey I, Ribeiro A, Deville A, Coz C, Michiels JF, Lyonnet S, Amiel J, Delattre O. Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. Cancer Lett. 2005;228:51–8.
7. Mosse YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, Laureys G, Speleman F, Kim C, Hou C, Hakonarson H, Torkamani A, Schork NJ, Brodeur GM, Tonini GP, Rappaport E, Devoto M, Maris JM. Identification of ALK as a major Familial neuroblastoma predisposition gene. Nature. 2008;455:930–5.
8. De Roos AJ, Olshan AF, Teschke K, Poole C, Savitz DA, Blatt J, Bondy ML, Pollock BH. Parental occupational exposures to chemicals and incidence of neuroblastoma in offspring. Am J Epidemiol. 2001;154:106–14.
9. De Roos AJ, Teschke K, Savitz DA, Poole C, Grufferman S, Pollock BH, Olshan AF. Parental occupational exposures to electromagnetic fields and radiation and the incidence of neuroblastoma in offspring. Epidemiology. 2001;12:508–17.
10. Tsubota S, Kadomatsu K. Origin and initiation mechanisms of neuroblastoma. Cell Tissue Res. 2018;372:211–21.
11. Maris JM, Mosse YP, Bradfield JP, Hou C, Monni S, Scott RH, Asgharzadeh S, Attiyeh EF, Diskin SJ, Laudenslager M, Winter C, Cole KA, Glessner JT, Kim C, Frackelton EC, Casalunovo T, Eckert AW, Capasso M, Rappaport EF, McConville C, London WB, Seeger RC, Rahman N, Devoto M, Grant SF, Li H, Hakonarson H. Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. N Engl J Med. 2008;358:2585–93.
12. Capasso M, Devoto M, Hou C, Asgharzadeh S, Glessner JT, Attiyeh EF, Mosse YP, Kim C, Diskin SJ, Cole KA, Bosse K, Diamond M, Laudenslager M, Winter C, Bradfield JP, Scott RH, Jagannathan J, Garris M, McConville C, London WB, Seeger RC, Grant SF, Li H, Rahman N, Rappaport E, Hakonarson H, Maris JM. Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. Nat Genet. 2009;41:718–23.
13. Nguyen le B, Diskin SJ, Capasso M, Wang K, Diamond MA, Glessner J, Kim C, Attiyeh EF, Mosse YP, Cole K, Iolascon A, Devoto M, Hakonarson H, Li HK, Maris JM. Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility loci. PLoS Genet. 2011;7:e1002026.
14. Wang K, Diskin SJ, Zhang H, Attiyeh EF, Winter C, Hou C, Schnepp RW, Diamond M, Bosse K, Mayes PA, Glessner J, Kim C, Frackelton E, Garris M, Wang Q, Glaberson W, Chiavacci R, Nguyen L, Jagannathan J, Saeki N, Sasaki H, Grant SF, Iolascon A, Mosse YP, Cole KA, Li H, Devoto M, McGrady PW, London WB, Capasso M, Rahman N, Hakonarson H, Maris JM. Integrative genomics identifies LMO1 as a neuroblastoma oncogene. Nature. 2011;469:216–20.
15. Diskin SJ, Capasso M, Schnepp RW, Cole KA, Attiyeh EF, Hou C, Diamond M, Carpenter EL, Winter C, Lee H, Jagannathan J, Latorre V, Iolascon A, Hakonarson H, Devoto M, Maris JM. Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. Nat Genet. 2012;44:1126–30.
16. McDaniel LD, Conkrite KL, Chang X, Capasso M, Vaksman Z, Oldridge DA, Zachariou A, Horn M, Diamond M, Hou C, Iolascon A, Hakonarson H, Rahman N, Devoto M, Diskin SJ. Common variants upstream of MLF1 at 3q25 and within CPZ at 4p16 associated with neuroblastoma. PLoS Genet. 2017;13:e1006787.
17. Capasso M, McDaniel LD, Cimmino F, Cirino A, Formicola D, Russell MR, Raman P, Cole KA, Diskin SJ. The functional variant rs34330 of CDKN1B is associated with risk of neuroblastoma. J Cell Mol Med. 2017;21:3224–30.
18. He J, Wang F, Zhu J, Zhang R, Yang T, Zou Y, Xia H. Association of potentially functional variants in the XPG gene with neuroblastoma risk in a Chinese population. J Cell Mol Med. 2016;20:1481–90.
19. Capasso M, Diskin S, Cimmino F, Acierno G, Totaro F, Petrosino G, Pezone L, Diamond M, McDaniel L, Hakonarson H, Iolascon A, Devoto M, Maris JM. Common genetic variants in NEFL influence gene expression and neuroblastoma risk. Cancer Res. 2014;74:6913–24.
20. Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J. Amplified DNA with limited homology to Myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature. 1983;305:245–8.

21. Masso-Valles D, Soucek L. Blocking Myc to treat cancer: reflecting on two decades of omomyc. Cells. 2020;9:883.
22. Yoda H, Inoue T, Shinozaki Y, Lin J, Watanabe T, Koshikawa N, Takatori A, Nagase H. Direct targeting of MYCN gene amplification by Site-Specific DNA alkylation in neuroblastoma. Cancer Res. 2019;79:830–40.
23. Guglielmi L, Cinnella C, Nardella M, Maresca G, Valentini A, Mercanti D, Felsani A, D'Agnano I. MYCN gene expression is required for the onset of the differentiation programme in neuroblastoma cells. Cell Death Dis. 2014;5:e1081.
24. Kramer M, Ribeiro D, Arsenian-Henriksson M, Deller T, Rohrer H. Proliferation and survival of embryonic sympathetic neuroblasts by MYCN and activated ALK signaling. J Neurosci. 2016;36:10425–39.
25. Petroni M, Veschi V, Prodosmo A, Rinaldo C, Massimi I, Carbonari M, Dominici C, McDowell HP, Rinaldi C, Screpanti I, Frati L, Bartolazzi A, Gulino A, Soddu S, Giannini G. MYCN sensitizes human neuroblastoma to apoptosis by HIPK2 activation through a DNA damage response. Mol Cancer Res. 2011;9:67–77.
26. Beckers A, Van Peer G, Carter DR, Gartlgruber M, Herrmann C, Agarwal S, Helsmoortel HH, Althoff K, Molenaar JJ, Cheung BB, Schulte JH, Benoit Y, Shohet JM, Westermann F, Marshall GM, Vandesompele J, De Preter K, Speleman F. MYCN-driven regulatory mechanisms controlling LIN28B in neuroblastoma. Cancer Lett. 2015;366:123–32.
27. Stermann A, Huebener N, Seidel D, Fest S, Eschenburg G, Stauder M, Schramm A, Eggert A, Lode HN. Targeting of MYCN by means of DNA vaccination is effective against neuroblastoma in mice. Cancer Immunol Immunother. 2015;64:1215–27.
28. Cohn SL, Tweddle DA. MYCN amplification remains prognostically strong 20 years after its clinical debut. Eur J Cancer. 2004;40:2639–42.
29. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, Hammond D. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med. 1985;313:1111–6.
30. Lin L, Deng C, Zhou C, Zhang X, Zhu J, Liu J, Wu H, He J. NSUN2 gene rs13181449 C > T polymorphism reduces neuroblastoma risk. Gene. 2023;854:147120.
31. Guan Q, Zhang X, Liu J, Zhou C, Zhu J, Wu H, Zhuo Z, He J. ALKBH5 gene polymorphisms and risk of neuroblastoma in Chinese children from Jiangsu Province. Cancer Innov. 2024;3:e103.
32. Zhou H, Zhuo Z, Chen S, Zhao J, Mo Y, Zhang J, He J, Ruan J. Polymorphisms in MYCN gene and neuroblastoma risk in Chinese children: a 3-center case-control study. Cancer Manag Res. 2018;10:1807–16.
33. Chen YP, Liao YX, Zhuo ZJ, Yuan L, Lin HR, Miao L, Li X, Huang XK, Zhou JY, Bian J, He J. Association between genetic polymorphisms of base excision repair pathway and glioma susceptibility in Chinese children. World J Pediatr. 2022;18:632–5.
34. Guan Q, Lin H, Hua W, Lin L, Liu J, Deng L, Zhang J, Cheng J, Yang Z, Li Y, Bian J, Zhou H, Li S, Li L, Miao L, Xia H, He J, Zhuo Z. Variant rs8400 enhances ALKBH5 expression through disrupting miR-186 binding and promotes neuroblastoma progression. Chin J Cancer Res. 2023;35:140–62.
35. Yin H, Wang X, Zhang S, He S, Zhang W, Lu H, Wang Y, He J, Zhou C. Nucleotide excision repair gene polymorphisms and hepatoblastoma susceptibility in Eastern Chinese children: A five-center case-control study. Chin J Cancer Res. 2024;36:298–305.
36. Lin L, Wang B, Zhang X, Deng C, Zhou C, Zhu J, Wu H, He J. Functional TET2 gene polymorphisms increase the risk of neuroblastoma in Chinese children. IUBMB Life. 2024;76:200–11.
37. Huang M, Weiss WA, Neuroblastoma. MYCN Cold Spring Harb Perspect Med. 2013;3:a014415.
38. Koumarianou A, Oikonomopoulou P, Baka M, Vlachodimitropoulos D, Argentos S, Piperos T, Christodoulou MI, Theodoulou K, Mariolis-Sapsakos T. Implications of the Incidental Finding of a MYCN Amplified Adrenal Tumor: A Case Report and Update of a Pediatric Disease Diagnosed in Adults. Case Rep Oncol Med. 2013; 2013: 393128.
39. Campbell K, Gastier-Foster JM, Mann M, Naranjo AH, Van Ryn C, Bagatell R, Matthay KK, London WB, Irwin MS, Shimada H, Granger MM, Hogarty MD, Park JR, DuBois. SG. Association of MYCN copy number with clinical features, tumor biology, and outcomes in neuroblastoma: A report from the children's oncology group. Cancer. 2017;123:4224–35.
40. Dzieran J, Rodriguez Garcia A, Westermark UK, Henley AB, Eyre Sanchez E, Trager C, Johansson HJ, Lehtio J, Arsenian-Henriksson M. MYCN-amplified neuroblastoma maintains an aggressive and undifferentiated phenotype by deregulation of Estrogen and NGF signaling. Proc Natl Acad Sci U S A. 2018;115:E1229–38.
41. Zhang P, Wu X, Basu M, Dong C, Zheng P, Liu Y, Sandler AD. MYCN amplification is associated with repressed cellular immunity in neuroblastoma: an in Silico immunological analysis of TARGET database. Front Immunol. 2017;8:1473.
42. Zhong ZY, Shi BJ, Zhou H, Wang WB. CD133 expression and MYCN amplification induce chemoresistance and reduce average survival time in pediatric neuroblastoma. J Int Med Res. 2018;46:1209–20.
43. Tanaka N, Fukuzawa M. MYCN downregulates integrin alpha1 to promote invasion of human neuroblastoma cells. Int J Oncol. 2008;33:815–21.
44. Rouah E, Wilson DR, Armstrong DL, Darlington GJ. N-myc amplification and neuronal differentiation in human primitive neuroectodermal tumors of the central nervous system. Cancer Res. 1989;49:1797–801.
45. Janardhanan R, Banik NL, Ray SK. N-Myc down regulation induced differentiation, early cell cycle exit, and apoptosis in human malignant neuroblastoma cells having wild type or mutant p53. Biochem Pharmacol. 2009;78:1105–14.
46. Zaatiti H, Abdallah J, Nasr Z, Khazen G, Sandler A, Abou-Antoun TJ. Tumorigenic proteins upregulated in the MYCN-amplified IMR-32 human neuroblastoma cells promote proliferation and migration. Int J Oncol. 2018;52:787–803.
47. Brandetti E, Veneziani I, Melaiu O, Pezzolo A, Castellano A, Boldrini R, Ferretti E, Fruci D, Moretta L, Pistoia V, Locatelli F, Cifaldi L. MYCN is an immunosuppressive oncogene dampening the expression of ligands for NK-cell-activating receptors in human high-risk neuroblastoma. Oncoimmunology. 2017;6:e1316439.
48. Dahlin AM, Hollegaard MV, Wibom C, Andersson U, Hougaard DM, Deltour I, Hjalmars U, Melin B. CCND2, CTNNB1, DDX3X, GLI2, SMARCA4, MYC, MYCN, PTCH1, TP53, and MLL2 gene variants and risk of childhood Medulloblastoma. J Neurooncol. 2015;125:75–8.
49. Huang X, Zhao J, Zhu J, Chen S, Fu W, Tian X, Lou S, Ruan J, He J, Zhou H. MYCN gene polymorphisms and Wilms tumor susceptibility in Chinese children. J Clin Lab Anal. 2019;33:e22988.

Publisher's note