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Polymorphisms in *MYCN* gene and neuroblastoma risk in Chinese children: a 3-center case–control study

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Introduction: Neuroblastoma is an embryonal tumor of the sympathetic nervous system. The *MYCN* oncogene is amplified in some neuroblastoma patients and correlated with poor prognosis. However, less is known regarding the relationship between *MYCN* gene single-nucleotide polymorphisms (SNPs) and neuroblastoma risk.

Patients and methods: To investigate the contribution of *MYCN* gene polymorphisms to neuroblastoma risk, we performed a 3-center case–control study by genotyping 4 SNPs in the *MYCN* gene from 429 cases and 884 controls.

Results: The results showed that only rs57961569 G>A was associated with neuroblastoma risk (GA vs GG: adjusted odds ratio =0.76, 95% confidence interval =0.60–0.98, *P*=0.033), while the other 3 SNPs were not (rs9653226 T>C, rs13034994 A>G, and rs60226897 G>A). Stratified analysis revealed that rs57961569 GG carriers were more likely to develop neuroblastoma in the following subgroups: children older than 18 months, tumor derived from the adrenal gland, and clinical stages III + IV. The increased neuroblastoma risk associated with the rs9653226 variant CC genotypes was more evident in the following subgroups: females, tumor derived from the adrenal gland, and clinical stages III + IV. The presence of 2–3 risk genotypes had a significant relationship with the following subgroups: tumor derived from the adrenal gland and clinical stages III + IV.

Conclusion: This study demonstrates a weak impact of *MYCN* gene polymorphisms on neuroblastoma risk, which should be further validated.

Keywords: neuroblastoma, susceptibility, *MYCN*, polymorphism

Introduction

Neuroblastoma, the most common extracranial solid tumor in childhood, develops from neural crest progenitor cells.[1,2] Neuroblastoma accounts for ~7% of all childhood malignancies, yet is responsible for 15% of all pediatric oncology deaths.[3–5] The prognosis of neuroblastoma patients is widely variable. Some patients may spontaneously regress without chemotherapy, while some relapse with therapy-resistant disease.[6] Neuroblastoma cases are generally classified into low-, intermediate-, and high-risk groups.[7] Among them, high-risk neuroblastoma patients comprise ~50% of all neuroblastoma cases.[8] The 5-year survival rates of high-risk neuroblastoma cases seldom exceed 40% despite intensive, multimodal therapy.[9] The difficulty in treating these high-risk neuroblastoma cases might be the distant metastasis of tumors to bone marrow.[7,10]

The etiology of neuroblastoma has been partly elucidated but remains obscure. Previous studies have indicated that environmental factors may influence risk of neu-

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roblastoma; however, the theory lacks direct evidence.[11,12] Growing attention has been directed to genetic and gene–environmental factors as underlying risks of neuroblastoma. Currently, numerous genetic variants have been determined to play critical roles in neuroblastoma carcinogenesis. For example, *PHOX2B*[13,14] and *ALK*[15,16] gene mutations were found in some neuroblastoma cases. Genome-wide association studies have also identified polymorphisms located in *TP53*, *HACE1*, *BARD1*, *LIN28B*, *LMO1*, and *CASC15* genes associated with neuroblastoma risk.[1,17,18]

MYCN, a member of the MYC protooncogene family, was first identified in neuroblastoma cell lines.[19] *MYCN* is a critical regulator of various cellular processes, including proliferation, differentiation, and apoptosis.[20–23] Moreover, aberration expression of *MYCN* is associated with tumor initiation and progression.[24–26] Amplification of *MYCN* is present in ~20% of neuroblastoma cases.[27] *MYCN* is closely associated with aggressive tumor type and poor prognosis.[28,29] Therefore, exploring the role of *MYCN* in carcinogenesis remains a research hotspot. However, few studies have been proposed to evaluate the association of *MYCN* gene polymorphisms and neuroblastoma risk. Given the importance of the *MYCN* gene in cancer initiation and development, we intend to explore whether *MYCN* gene polymorphisms could predispose a patient to neuroblastoma risk.

Patients and methods

Participants

A total of 429 neuroblastoma cases and 884 healthy controls from 3 centers were included in this study.[30–32] All of the cases were newly confirmed and histopathologically diagnosed neuroblastoma patients without progressive disease or previous treatments. The controls were age-, gender-, and ethnicity-matched to cases and were randomly recruited from children undergoing routine medical examination at the same hospital during the same period. All of the enrolled subjects were of Chinese Han ethnicity. More specifically, 36 cases and 72 controls were enrolled from The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (Table S1), 275 cases and 531 controls were enrolled from Guangzhou Women and Children's Medical Center, and the remaining 118 cases and 281 controls were recruited from The First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all of the participants' parents. Details of the selection process of the included participants were provided in our previous publication.[33–35] The institutional review board of the above-mentioned 3 hospitals approved the study.

Single-nucleotide polymorphism (SNP) selection and genotyping

We chose potentially functional SNPs of interest from the dbSNP database (http://www.ncbi.nlm.nih.gov/) based on the following criteria: 1) minor allele frequency >5% for Chinese Han subjects; 2) potentially functional, such as affecting the binding capacity of transcription factor or microRNA binding sites, or leading to amino acid alterations. We chose 4 potentially functional SNPs in the *MYCN* gene (rs57961569 G>A, rs9653226 T>C, rs13034994 A>G, and rs60226897 G>A) for analysis. As predicted by SNPinfo (http://snpinfo.niehs.nih.gov/), all of them were located in the transcription factor binding sites. Moreover, they all had a minor allele frequency >5% in Chinese Han subjects. As shown in Figure S1, there was no significant linkage disequilibrium (R^2<0.8) among these 4 SNPs (R^2=0.220 between rs57961569 and rs9653226; R^2=0.185 between rs57961569 and rs13034994; R^2=0.103 between rs57961569 and rs60226897; R^2=0.348 between rs9653226 and rs13034994; R^2=0.161 between rs9653226 and rs60226897; R^2=0.500 between rs13034994 and rs6022689). First, the genomic DNA was extracted from the peripheral blood donated by subjects. The *MYCN* gene polymorphisms were detected using TaqMan real-time PCR, as described elsewhere.[36–39] Eight blank wells containing water as negative controls were also placed in each 384-well plate as the means of quality control. In addition, we randomly regenotyped 10% of the samples and got a concordance rate of 100%.

Statistical analysis

Testing of Hardy–Weinberg equilibrium for the selected polymorphisms in controls was performed using a goodness-of-fit χ^2 test. A 2-sided χ^2 test was used for comparisons of 2 groups in allele frequencies and demographic variables. We evaluated the associations between genotypes and neuroblastoma risk using odds ratios (ORs) and 95% confidence intervals (CIs) calculated from logistic regression analysis. Risk genotypes were rs57961569 GG, rs9653226 CC, rs13034994 GG, and rs60226897 GG; covariates, including age and gender, were used for adjustment. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). *P*-values <0.05 were considered significant.

Results

Population characteristics

The population demographics for the Guangzhou and Zhengzhou subjects were presented in our former publication.[30–32] The population demographics for the Wenzhou subjects are

shown in Table S1, while those of the combined subjects are shown in Table S2. No statistically significant differences were observed between neuroblastoma cases and controls regarding age (*P*=0.229, *P*=0.484, *P*=0.496, *P*=0.119) and gender (*P*=0.510, *P*=0.196, *P*=1.000, *P*=0.840) for the Guangdong, Henan, Wenzhou, and combined subjects, respectively.

Correlation of MYCN gene polymorphisms with neuroblastoma susceptibility

The genotype frequencies of *MYCN* gene polymorphisms and neuroblastoma susceptibility between all cases and controls for combined subjects and divided subjects are shown in Tables 1 and S3, respectively. All of the 4 genotyped *MYCN* SNPs in controls conformed to the Hardy–Weinberg equilibrium for combined subjects (rs57961569 G>A, *P*=0.379; rs9653226 T>C, *P*=0.569; rs13034994 A>G, *P*=0.907; and rs60226897 G>A, *P*=0.526). In single genotype analysis, only 1 SNP rs57961569 G>A was associated with neuroblastoma risk (GA vs GG: adjusted OR [AOR)] =0.76, 95% CI =0.60–0.98, *P*=0.033). No statistically significant associations were found between the other 3 SNPs (rs9653226 T>C, rs13034994 A>G, and rs60226897 G>A) and neuroblastoma risk. We found that compared with subjects carrying 0–1 risk genotypes, subjects carrying 2–3 combined risk genotypes

Table 1 Genotype frequencies of *MYCN* gene polymorphisms and neuroblastoma susceptibility

Genotype	Cases (N=429)	Controls (N=884)	*P*-value[a]	Crude OR (95% CI)	*P*-value	AOR (95% CI)[b]	*P*-value[b]
rs57961569 G>A (HWE =0.379)							
GG	202 (47.09)	372 (42.08)		1.00		1.00	
GA	171 (39.86)	412 (46.61)		**0.76 (0.60–0.98)**	**0.033**	**0.76 (0.60–0.98)**	**0.033**
AA	56 (13.05)	100 (11.31)		1.03 (0.71–1.49)	0.870	1.03 (0.71–1.50)	0.862
Additive			0.069	0.93 (0.78–1.11)	0.411	0.93 (0.78–1.11)	0.414
Dominant	227 (52.91)	512 (57.92)	0.086	0.82 (0.64–1.03)	0.087	0.82 (0.65–1.03)	0.087
Recessive	373 (86.95)	784 (88.69)	0.360	1.18 (0.83–1.67)	0.361	1.18 (0.83–1.67)	0.355
rs9653226 T>C (HWE =0.569)							
TT	138 (32.17)	293 (33.14)		1.00		1.00	
TC	202 (47.09)	439 (49.66)		0.98 (0.75–1.27)	0.862	0.98 (0.75–1.27)	0.850
CC	89 (20.75)	152 (17.19)		1.24 (0.89–1.73)	0.197	1.24 (0.89–1.73)	0.201
Additive			0.292	1.10 (0.93–1.29)	0.272	1.10 (0.93–1.29)	0.278
Dominant	291 (67.83)	591 (66.86)	0.724	1.05 (0.82–1.34)	0.724	1.04 (0.82–1.34)	0.736
Recessive	340 (79.25)	732 (82.81)	0.119	1.26 (0.94–1.69)	0.119	1.26 (0.94–1.69)	0.121
rs13034994 A>G (HWE =0.907)							
AA	265 (61.77)	526 (59.50)		1.00		1.00	
AG	134 (31.24)	311 (35.18)		0.86 (0.67–1.10)	0.221	0.86 (0.67–1.10)	0.225
GG	30 (6.99)	47 (5.32)		1.27 (0.78–2.05)	0.335	1.27 (0.79–2.06)	0.329
Additive			0.228	0.98 (0.81–1.19)	0.868	0.99 (0.81–1.19)	0.878
Dominant	164 (38.23)	358 (40.50)	0.431	0.91 (0.72–1.15)	0.431	0.91 (0.72–1.15)	0.437
Recessive	399 (93.01)	837 (94.68)	0.225	1.34 (0.83–2.15)	0.227	1.34 (0.84–2.16)	0.222
rs60226897 G>A (HWE =0.526)							
GG	208 (48.48)	410 (46.38)		1.00		1.00	
GA	174 (40.56)	378 (42.76)		0.91 (0.71–1.16)	0.437	0.91 (0.71–1.16)	0.442
AA	47 (10.96)	96 (10.86)		0.97 (0.66–1.42)	0.857	0.97 (0.66–1.43)	0.871
Additive			0.738	0.96 (0.80–1.14)	0.610	0.96 (0.81–1.14)	0.623
Dominant	221 (51.52)	474 (53.62)	0.474	0.92 (0.73–1.16)	0.474	0.92 (0.73–1.16)	0.482
Recessive	382 (89.04)	788 (89.14)	0.958	1.01 (0.70–1.46)	0.958	1.01 (0.70–1.47)	0.946
Combined effect of risk genotypes[c]							
0	199 (46.39)	428 (48.42)		1.00		1.00	
1	37 (8.62)	107 (12.10)		0.74 (0.49–1.12)	0.157	0.74 (0.49–1.12)	0.151
2	87 (20.28)	173 (19.57)		1.08 (0.80–1.47)	0.617	1.08 (0.80–1.47)	0.620
3	106 (24.71)	176 (19.91)		1.30 (0.97–1.74)	0.084	1.29 (0.96–1.74)	0.086
Trend			0.086	1.09 (0.99–1.19)	0.088	1.08 (0.99–1.19)	0.090
0–1	236 (55.01)	535 (60.52)		1.00		1.00	
2–3	193 (44.99)	349 (39.48)	0.057	1.25 (0.99–1.58)	0.057	1.25 (0.99–1.58)	0.058

Notes: [a]χ^2 test for genotype distributions between neuroblastoma patients and controls. [b]Adjusted for age and gender. [c]Risk genotypes were rs57961569 GG, rs9653226 CC, rs13034994 GG, and rs60226897 GG. Bold figures indicate 95% CI excluded 1 or *P*<0.05.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

of *MYCN* exhibited enhanced risk of neuroblastoma, but this finding was nonsignificant (AOR =1.25; 95% CI =0.99–1.58).

Stratification analysis

Stratification analysis was further conducted to evaluate the effects of different strata (age, gender, tumor sites of origin, and clinical stages) on the association between the selected polymorphisms and neuroblastoma risk (Table 2). Concerning the rs57961569 polymorphism, significant association was detected in the following subgroups: children older than 18 months (GG vs GA: AOR =1.38; 95% CI =1.01–1.88, *P*=0.040), tumor derived from the adrenal gland (GG vs GA: AOR =1.88; 95% CI =1.30–2.72, *P*=0.0008), and clinical stages III + IV (GG vs GA: AOR =1.40, 95% CI =1.02–1.92, *P*=0.037). The conferred increased neuroblastoma risk associated with the rs9653226 variant CC genotypes was more evident in the following subgroups: females (AOR =1.63, 95% CI =1.07–2.48, *P*=0.023), tumor derived from the adrenal gland (AOR =1.79, 95% CI =1.21–2.63, *P*=0.003), and clinical stages III + IV (AOR =1.63, 95% CI =1.15–2.31, *P*=0.006). In the stratified analysis of the cumulative effects of risk genotypes, we found that the presence of 2–3 risk genotypes had a significant relationship with the following subgroups: tumor derived from the adrenal gland (AOR =1.66, 95% CI =1.18–2.32, *P*=0.003) and clinical stages III + IV (AOR =1.35, 95% CI =1.004–1.81, *P*=0.047).

Discussion

In the current study, we explored the impact of SNPs of the *MYCN* gene on the risk of neuroblastoma in the Chinese population. Our data revealed that the rs57961569 G>A polymorphism in the *MYCN* gene presented significant inverse associations with neuroblastoma risk.

MYCN is located on chromosome 2p24.3 and encodes a pleiotropic nuclear phosphoprotein. The encoding protein consists of 2 domains: a carboxy-terminal DNA-binding and protein interaction domain and an amino-terminal transcriptional activation domain. In healthy conditions in humans and mice, *MYCN* expression is high in certain tissues in the developing embryo, while it is low or even absent in adult tissues.[40,41] Amplification of *MYCN* could promote proliferation and cell cycle progression. *MYCN* could also enhance neuroblastoma cell migration and invasion through downregulation of integrins α1 and β1.[42,43] Moreover, *MYCN* modulates antigens on the surface of tumor cells, thus influencing immune surveillance.[44] Brandetti et al[45] demonstrated that *MYCN* functions as an immunosuppressive oncogene in neuroblastoma cells by negatively regulating the expression

Table 2 Stratification analysis of risk genotypes with neuroblastoma susceptibility

Variables	rs57961569 (cases/controls)		AOR (95% CI)	*P*-value[a]	rs9653226 (cases/controls)		AOR (95% CI)	*P*-value[a]	Combined		AOR (95% CI)	*P*-value[a]
	GA	GG			TT/TC	CC			0–1	2–3		
Age, month												
≤18	59/156	67/149	1.19 (0.78–1.80)	0.419	112/274	34/66	1.25 (0.78–2.00)	0.347	80/200	66/140	1.18 (0.80–1.74)	0.408
>18	112/256	135/223	**1.38 (1.01–1.88)**	**0.040**	228/458	55/86	1.29 (0.88–1.87)	0.189	156/335	127/209	1.31 (0.98–1.75)	0.073
Gender												
Female	72/181	86/156	1.38 (0.94–2.02)	0.098	136/308	49/68	**1.63 (1.07–2.48)**	**0.023**	102/230	83/146	1.28 (0.90–1.83)	0.172
Male	99/231	116/216	1.24 (0.90–1.73)	0.190	204/424	40/84	0.99 (0.66–1.50)	0.971	134/305	110/203	1.23 (0.90–1.68)	0.189
Sites of origin												
Adrenal gland	53/412	89/372	**1.88 (1.30–2.72)**	**0.0008**	120/732	44/152	**1.79 (1.21–2.64)**	**0.003**	79/535	85/349	**1.66 (1.18–2.32)**	**0.003**
Retroperitoneal	44/412	41/372	1.02 (0.65–1.60)	0.936	76/732	20/152	1.27 (0.76–2.15)	0.365	56/535	40/349	1.09 (0.71–1.67)	0.706
Mediastinum	49/412	55/372	1.25 (0.83–1.89)	0.281	105/732	18/152	0.82 (0.48–1.39)	0.455	70/535	53/349	1.17 (0.80–1.71)	0.423
Others	23/412	11/372	0.53 (0.25–1.10)	0.086	34/732	4/152	0.57 (0.20–1.62)	0.291	29/535	9/349	0.48 (0.22–1.02)	0.055
Clinical stages												
I + II + 4s	76/412	83/372	1.22 (0.87–1.71)	0.260	152/732	27/152	0.84 (0.54–1.32)	0.453	101/535	78/349	1.19 (0.86–1.65)	0.292
III + IV	88/412	110/372	**1.40 (1.02–1.92)**	**0.037**	170/732	57/152	**1.63 (1.15–2.31)**	**0.006**	121/535	106/349	**1.35 (1.004–1.81)**	**0.047**

Notes: [a]Adjusted for age and gender. Bold figures indicate 95% CI excluded 1 or *P*<0.05.
Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.

of ligands for DNAM-1and NKG2D NK-cell-activating receptors. Importantly, a study conducted by Dahlin et al[46] was the first to investigate the relationship between *MYCN* gene variants and cancer risk. The study failed to establish a significant relationship between *MYCN* gene variant rs922 G>A and medulloblastoma risk from 243 cases and 247 controls of Swedish and Danish children. The significance of *MYCN* in the initiation and development of cancer is self-evident.

Herein, we investigated for the first time whether *MYCN* gene SNPs could affect the risk of neuroblastoma in Chinese children. Among the 4 analyzed SNPs, only rs57961569 G>A was associated with neuroblastoma risk. In the combined analysis, subjects carrying 2–3 risk genotypes tended to have increased neuroblastoma risk in comparison to those with 0–1 risk genotypes. This phenomenon was quite biologically plausible, as a single polymorphism in each gene may not be strong enough to influence the risk of cancer. Intriguingly, stratified analysis showed that individuals harboring the rs57961569 GG alleles were more likely to have a tumor in the following subgroups: children older than 18 months, tumor derived from the adrenal gland, and clinical stages III + IV. The contributing role of the rs9653226 CC genotype to increasing neuroblastoma risk was more evident in the females, tumor derived from the adrenal gland, and clinical stages III + IV subgroups. In the stratified analysis of the cumulative effects of risk genotypes, we found that the presence of 2–3 risk genotypes had a positive relationship with the following subgroups: tumor derived from the adrenal gland and clinical stages III + IV. On one hand, the same polymorphism might have a different role in cancer risk, depending on different ethnicities, regions, and cancer types. On the other hand, a conflicting role might also be the small sample size in the stratified analysis.

Several limitations accompany the merits of this study. First, the sample size was relatively small, although we gathered samples from 3 centers, especially for the stratification analysis. As a result, the strength of the statistical power may not be strong enough. Second, being a retrospective investigation, only genetic information in the *MYCN* gene was measured. Measuring other environmental factors, such as childhood exposure, dietary habits, and health situation, is critical in helping to further elucidate the role of *MYCN* polymorphisms in neuroblastoma risk. Third, only 4 SNPs in the *MYCN* gene were analyzed, so more polymorphisms in the *MYCN* gene should be investigated.

Overall, in the Chinese population studied, we provide the first evidence that polymorphisms in *MYCN* gene could influence neuroblastoma risk in a low-impact manner. Larger sample size studies with additional functional analysis are needed to better elucidate the role of *MYCN* polymorphisms in neuroblastoma risk.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

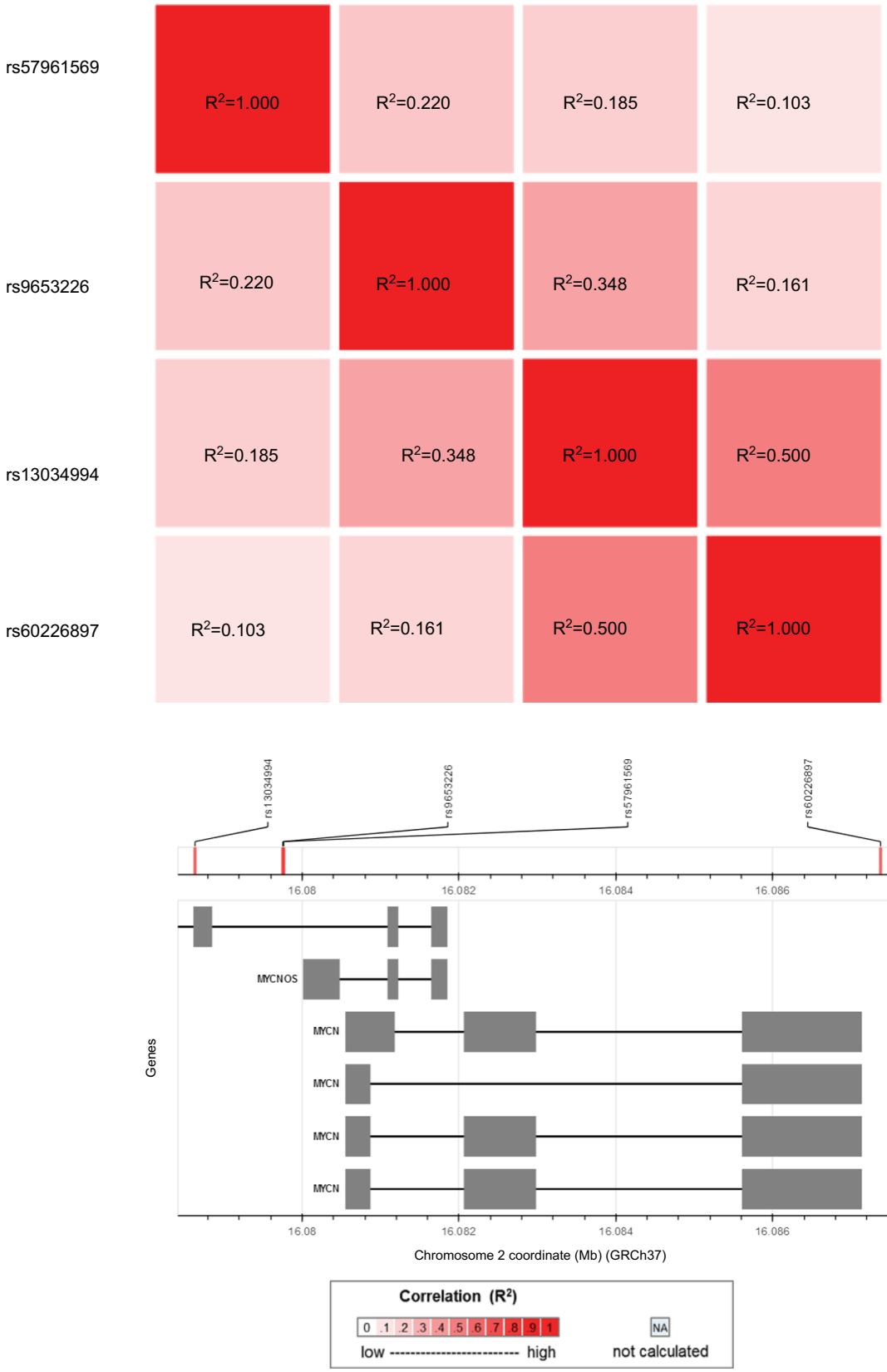

Figure S1 Linkage disequilibrium analysis for the 4 selected polymorphisms in *MYCN* gene in Han Chinese population consisting of CHB (Han Chinese in Beijing, China) and CHS (Southern Han Chinese) subjects.

Table S1 Frequency distribution of demographic characteristics for Wenzhou subjects

Variables	Cases (N=36)		Controls (N=72)		*P*-value[a]
	Number	%	Number	%	
Age range, month	0.05–72		8–72		0.496
Mean ± SD	20.25±20.73		23.58±15.36		
≤18	20	55.56	35	48.61	
>18	16	44.44	37	51.39	
Gender					1.000
Female	17	47.22	34	47.22	
Male	19	52.78	38	52.78	
Clinical stages					
I	15	41.67	–	–	
II	2	5.56	–	–	
III	9	25.00	–	–	
IV	7	19.44	–	–	
4s	3	8.33	–	–	
Sites of origin					
Adrenal gland	11	30.56	–	–	
Retroperitoneal region	9	25.00	–	–	
Mediastinum	14	38.89	–	–	
Other regions	2	5.56	–	–	

Notes: [a]Two-sided χ^2 test for distributions between neuroblastoma patients and controls. "–" indicates no value.

Table S2 Frequency distribution of demographic characteristics for combined subjects

Variables	Cases (N=429)		Controls (N=884)		*P*-value[a]
	Number	%	Number	%	
Age range, month	0.00–132		0.07–156		0.119
Mean ± SD	34.61±27.49		34.08±28.25		
≤18	146	34.03	340	38.46	
>18	283	65.97	544	61.54	
Gender					0.840
Female	185	43.12	376	42.53	
Male	244	56.88	508	57.47	
Clinical stages					
I	84	19.58	–	–	
II	95	22.14	–	–	
III	77	17.95	–	–	
IV	150	34.97	–	–	
4s	14	3.26	–	–	
NA	9	2.10			
Sites of origin					
Adrenal gland	164	38.23	–	–	
Retroperitoneal region	96	22.38	–	–	
Mediastinum	123	28.67	–	–	
Other regions	38	8.86	–	–	
NA	8	1.86			

Notes: [a]Two-sided χ^2 test for distributions between neuroblastoma patients and controls. "–" indicates no value.
Abbreviation: NA, not available.

Table S3 Genotype frequencies of *MYCN* gene polymorphisms and neuroblastoma susceptibility (divided subjects)

Genotype	Guangdong province				Henan province				Wenzhou subjects			
	Cases (N=275)	Controls (N=531)	AOR (95% CI)[a]	*P*-value[a]	Cases (N=118)	Controls (N=281)	AOR (95% CI)[a]	*P*-value[a]	Cases (N=36)	Controls (N=72)	AOR (95% CI)[a]	*P*-value[a]
rs57961569 G>A												
GG	124 (45.09)	230 (43.31)	1.00		64 (54.24)	113 (40.21)	1.00		14 (38.89)	29 (40.28)	1.00	
GA	114 (41.45)	243 (45.76)	0.87 (0.64–1.19)	0.371	42 (35.59)	135 (48.04)	0.54 (0.34–0.86)	0.009	15 (41.67)	34 (47.22)	0.93 (0.38–2.28)	0.877
AA	37 (13.45)	58 (10.92)	1.19 (0.75–1.90)	0.459	12 (10.17)	33 (11.74)	0.64 (0.31–1.34)	0.237	7 (19.44)	9 (12.50)	1.61 (0.48–5.35)	0.439
Additive			1.02 (0.82–1.26)	0.868			0.69 (0.49–0.97)	0.032			1.19 (0.66–2.14)	0.558
Dominant	151 (54.91)	301 (56.69)	0.93 (0.69–1.25)	0.626	54 (45.76)	168 (59.79)	0.56 (0.36–0.86)	0.009	22 (61.11)	43 (59.72)	1.07 (0.46–2.47)	0.876
Recessive	238 (86.55)	473 (89.06)	1.28 (0.82–1.99)	0.272	106 (89.83)	248 (88.26)	0.86 (0.43–1.74)	0.677	29 (80.56)	63 (87.50)	1.67 (0.56–5.01)	0.359
rs9653226 T>C												
TT	93 (33.82)	181 (34.09)	1.00		30 (25.42)	92 (32.74)	1.00		15 (41.67)	20 (27.78)	1.00	
TC	124 (45.09)	262 (49.34)	0.91 (0.66–1.27)	0.584	62 (52.54)	141 (50.18)	1.34 (0.81–2.23)	0.261	16 (44.44)	36 (50.00)	0.57 (0.23–1.41)	0.225
CC	58 (21.09)	88 (16.57)	1.29 (0.85–1.96)	0.228	26 (22.03)	48 (17.08)	1.61 (0.86–3.04)	0.140	5 (13.89)	16 (22.22)	0.40 (0.12–1.36)	0.142
Additive			1.10 (0.90–1.36)	0.354			1.28 (0.93–1.74)	0.129			0.62 (0.34–1.12)	0.115
Dominant	182 (66.18)	350 (65.91)	1.01 (0.74–1.37)	0.964	88 (74.58)	189 (67.26)	1.41 (0.87–2.29)	0.166	21 (58.33)	52 (72.22)	0.52 (0.22–1.22)	0.132
Recessive	217 (78.91)	443 (83.43)	1.36 (0.94–1.97)	0.101	92 (77.97)	233 (82.92)	1.34 (0.78–2.29)	0.293	31 (86.11)	56 (77.78)	0.56 (0.19–1.68)	0.299
rs13034994 A>G												
AA	166 (60.36)	300 (56.50)	1.00		77 (65.25)	176 (62.63)	1.00		22 (61.11)	50 (69.44)	1.00	
AG	90 (32.73)	202 (38.04)	0.80 (0.59–1.10)	0.171	32 (27.12)	89 (31.67)	0.84 (0.51–1.36)	0.472	12 (33.33)	20 (27.78)	1.41 (0.58–3.42)	0.444
GG	19 (6.91)	29 (5.46)	1.18 (0.64–2.17)	0.602	9 (7.63)	16 (5.69)	1.37 (0.58–3.26)	0.476	2 (5.56)	2 (2.78)	2.39 (0.31–18.51)	0.404
Additive			0.93 (0.73–1.19)	0.577			1.01 (0.70–1.44)	0.974			1.47 (0.72–3.00)	0.294
Dominant	109 (39.64)	231 (43.50)	0.85 (0.63–1.14)	0.284	41 (34.75)	105 (37.37)	0.91 (0.58–1.44)	0.698	14 (38.89)	22 (30.56)	1.50 (0.64–3.50)	0.348
Recessive	256 (93.09)	502 (94.54)	1.28 (0.70–2.33)	0.422	109 (92.37)	265 (94.31)	1.45 (0.6–3.41)	0.391	34 (94.44)	70 (97.22)	2.14 (0.28–16.16)	0.463
rs60226897 G>A												
GG	130 (47.27)	262 (49.34)	1.00		64 (54.24)	117 (41.64)	1.00		14 (38.89)	31 (43.06)	1.00	
GA	116 (42.18)	215 (40.49)	1.09 (0.80–1.48)	0.600	43 (36.44)	130 (46.26)	0.59 (0.37–0.94)	0.028	15 (41.67)	33 (45.83)	1.05 (0.43–2.57)	0.919
AA	29 (10.55)	54 (10.17)	1.09 (0.66–1.80)	0.727	11 (9.32)	34 (12.10)	0.60 (0.28–1.26)	0.176	7 (19.44)	8 (11.11)	1.98 (0.59–6.63)	0.270
Additive			1.06 (0.85–1.32)	0.604			0.70 (0.50–0.98)	0.037			1.32 (0.74–2.38)	0.350
Dominant	145 (52.73)	269 (50.66)	1.09 (0.81–1.46)	0.574	54 (45.76)	164 (58.36)	0.60 (0.39–0.92)	0.019	22 (61.11)	41 (56.94)	1.23 (0.53–2.85)	0.624
Recessive	246 (89.45)	477 (89.83)	1.05 (0.65–1.70)	0.835	107 (90.68)	247 (87.90)	0.76 (0.37–1.56)	0.453	29 (80.56)	64 (88.89)	1.93 (0.63–5.87)	0.248
Combined effect of risk genotypes[b]												
0	131 (47.64)	239 (45.01)	1.00		47 (39.83)	152 (54.09)	1.00		21 (58.33)	37 (51.39)	1.00	
1	24 (8.73)	78 (14.69)	0.56 (0.34–0.93)	0.025	12 (10.17)	22 (7.83)	1.83 (0.84–3.99)	0.130	1 (2.78)	7 (9.72)	0.23 (0.03–2.07)	0.191
2	53 (19.27)	111 (20.90)	0.86 (0.58–1.28)	0.458	26 (22.03)	49 (17.44)	1.79 (1.00–3.21)	0.049	8 (22.22)	13 (18.06)	1.10 (0.39–3.15)	0.859
3	67 (24.36)	103 (19.40)	1.20 (0.82–1.74)	0.349	33 (27.97)	58 (20.64)	1.84 (1.07–3.16)	0.027	6 (16.67)	15 (20.83)	0.66 (0.22–2.02)	0.469
Trend			1.04 (0.92–1.17)	0.520			1.24 (1.04–1.47)	0.014			0.92 (0.66–1.29)	0.629
0–1	155 (56.36)	317 (59.70)	1.00		59 (50.00)	174 (61.92)	1.00		22 (61.11)	44 (61.11)	1.00	
2–3	120 (43.64)	214 (40.30)	1.15 (0.85–1.54)	0.362	59 (50.00)	107 (38.08)	1.65 (1.07–2.55)	0.024	14 (38.89)	28 (38.89)	1.00 (0.43–2.31)	0.999

Notes: [a]Adjusted for age and gender. [b]Risk genotypes were rs57961569 GG, rs9653226 CC, rs13034994 GG, and rs60226897 GG.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.