

Association of Common Genetic Variants in Pre-microRNAs and Neuroblastoma Susceptibility: A Two-Center Study in Chinese Children

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Neuroblastoma is a commonly occurring extracranial pediatric solid tumor without defined etiology. Polymorphisms in pre-miRNAs have been demonstrated to associate with the risk of several cancers. So far, no such polymorphism has been investigated in neuroblastoma. With this in mind, we performed a two-center case-control study to assess the association of genetic variants in pre-miRNAs and neuroblastoma susceptibility in Chinese children, including 393 cases and 812 controls. We found that *miR-34b/c* rs4938723 T > C polymorphism was significantly associated with decreased neuroblastoma risk (TC versus TT: adjusted odds ratio [OR] = 0.51, 95% confidence interval [CI] = 0.39–0.67; TC/CC versus TT: adjusted OR = 0.62, 95% CI = 0.48–0.79). We also observed the significant association between the *miR-218* rs11134527 A > G polymorphism and decreased neuroblastoma risk (AG versus AA: adjusted OR = 0.73, 95% CI = 0.56–0.96). Stratified analysis further demonstrated that the protective effect of the rs4938723 T > C polymorphism remained prominent in the subgroups, regardless of age, gender, and clinical stages. In term of sites of origin, this polymorphism significantly reduced the risk of tumors originating from the adrenal gland. We further validated the significant results using false-positive report probability analyses. Overall, the *miR-34b/c* rs4938723 T > C and *miR-218* rs11134527 A > G polymorphisms displayed a protective role from neuroblastoma. These findings need further validation.

INTRODUCTION

Neuroblastoma is one of the most commonly occurring extracranial pediatric solid tumors, which accounts for approximately 8%–10% of all childhood cancers and 15% of pediatric malignancy deaths.¹ The 10-year survival rate in patients with low-risk neuroblastoma is around 90%, whereas the long-term survival of high-risk neuroblastoma remains less than 40%, despite great advances achieved in the treatment of cancers.^{2,3} Environment risk factors for developing neuroblastoma remain undefined.^{4,5} Numerous studies have indicated that genetic factors may play a critical role in the occurrence of neuroblastoma, such as *ALK* gene mutations^{6–8} and genome-wide-as-

sociation-study-identified susceptibility loci in the *CASC15*, *BARD1*, *DUSP12*, *DDX4*, *IL31RA*, *HSD17B12*, *LMO1*, *HACE1*, *LIN28B*, *MLF1*, and *CPZ* genes,^{9–14} as well as polymorphisms in the *FAS* and *FASL*,¹⁵ *XPG*¹⁶ genes.

MicroRNAs (miRNAs) are non-coding single-stranded RNAs of approximately 17–22 nt in length, which is one of the largest classes of gene regulators.¹⁷ They can bind to 3' UTR of mRNA to induce the degradation or translational inhibition of the corresponding mRNAs, consequently silencing target genes.¹⁸ In the nucleus, primary miRNA (pri-miRNA) transcripts with lengths from several hundred nucleotides to several kilobases can be cleaved to generate a precursor miRNA (pre-miRNA) of about 70 nt, which can fold to form a stem-loop intermediate.^{19,20} Next, the intermediate is further processed to produce a mature miRNA.¹⁹ Polymorphisms or mutations in the promoter or in the miRNAs sequence may lead to altered structure or expression of miRNA, thereby influencing the expression of hundreds of target genes.²¹ Polymorphisms in miRNAs may modify cancer susceptibility and prognosis.^{22–25} The association between genetic variants in pre-miRNAs and cancer susceptibility has been investigated in various types of cancer,²⁶ but not in neuroblastoma. Therefore, we performed a two-center case-control study to assess the association of genetic variants in pre-miRNAs and neuroblastoma susceptibility in Chinese children.

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Table 1. Logistic Regression Analyses on Associations between Selected Polymorphisms and Neuroblastoma Risk in Chinese Children

miRNA	SNP	Allele			Case (n = 393)			Control (n = 812)			Heterozygous (AB versus AA)			Dominant (AB/BB versus AA)			Recessive (BB versus AB/AA)			HWE
		A	B	C	AA	AB	BB	AA	AB	BB	AOR (95% CI) ^a	p Value ^a	AOR (95% CI) ^a	p Value ^a	AOR (95% CI) ^a	p Value ^a	AOR (95% CI) ^a	p Value ^a		
<i>miR-27a</i>	rs895819	T	C		220	153	20	442	312	58	0.99 (0.77–1.27)	0.913	0.94 (0.74–1.20)	0.623	0.70 (0.42–1.18)	0.184	0.772			
<i>miR-34b/c</i>	rs4938723	T	C		221	107	49	377	358	75	0.51 (0.39–0.67) ^b	<0.0001 ^b	0.62 (0.48–0.79) ^b	0.0001 ^b	1.46 (1.00–2.15)	0.052	0.448			
<i>miR-137</i>	rs1625579	T	G		343	46	3	719	90	1	1.07 (0.74–1.57)	0.717	1.13 (0.78–1.64)	0.519	6.23 (0.65–60.00)	0.114	0.290			
<i>miR-146a</i>	rs2910164	C	G		142	189	60	282	397	130	0.94 (0.72–1.23)	0.671	0.94 (0.73–1.20)	0.607	0.94 (0.68–1.32)	0.728	0.621			
<i>miR-149</i>	rs2292832	T	C		286	62	32	560	172	59	0.70 (0.51–0.97) ^b	0.032 ^b	0.79 (0.60–1.05)	0.106	1.14 (0.73–1.79)	0.559	<0.001			
<i>miR-196a2</i>	rs11614913	T	C		107	192	94	230	399	183	1.04 (0.78–1.39)	0.793	1.06 (0.81–1.39)	0.673	1.08 (0.81–1.43)	0.607	0.691			
<i>miR-218</i>	rs11134527	A	G		154	164	73	276	403	131	0.73 (0.56–0.96) ^b	0.022 ^b	0.80 (0.62–1.02)	0.073	1.19 (0.86–1.63)	0.290	0.425			
<i>miR-423</i>	rs6505162	C	A		244	132	17	522	258	31	1.09 (0.84–1.42)	0.497	1.10 (0.86–1.41)	0.448	1.13 (0.62–2.07)	0.694	0.900			
<i>miR-608</i>	rs4919510	G	C		127	190	76	227	405	179	0.84 (0.64–1.11)	0.217	0.81 (0.63–1.06)	0.122	0.84 (0.62–1.14)	0.263	0.948			

AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

^aAdjusted for age and gender.^bFor these values, the 95% CI excluded 1 or p < 0.05.

RESULTS

Characteristics of the Participants

The demographic and clinical characteristics data of neuroblastoma cases and cancer-free controls are summarized in [Table S1](#). No significant differences were observed between cases and controls for the Southern Chinese children regarding age ($p = 0.229$) and gender ($p = 0.510$) and the Northern Chinese children regarding age ($p = 0.484$) and gender ($p = 0.196$).

Association of Selected Polymorphisms with Neuroblastoma Risk

As shown in [Tables 1](#) and [S2](#), all of the nine selected polymorphisms ([Table S3](#)) were in accordance with Hardy-Weinberg equilibrium (HWE) in the controls, and the HWE p values ranged from 0.290 to 0.948, except for the *miR-149* rs2292832 T > C. As a result, this polymorphism was excluded from further analyses. Of the remaining eight polymorphisms, we found that the *miR-34b/c* rs4938723 T > C polymorphism was significantly associated with decreased neuroblastoma susceptibility (TC versus TT: adjusted odds ratio [OR] = 0.51, 95% confidence interval [CI] = 0.39–0.67; TC/CC versus TT: adjusted OR = 0.62, 95% CI = 0.48–0.79; and C versus T: adjusted OR = 0.82, 95% CI = 0.68–0.99) ([Figures 1, 2, and 3](#)). The *miR-218* rs11134527 A > G polymorphism was also shown to significantly decrease neuroblastoma susceptibility (AG versus AA: adjusted OR = 0.73, 95% CI = 0.56–0.96) ([Table S4](#)).

Stratified Analysis

We further explored the association of *miR-34b/c* rs4938723 T > C and *miR-218* rs11134527 A > G polymorphisms with neuroblastoma susceptibility by stratified analysis ([Table 2](#)). We found that the protective effect of the *miR-34b/c* rs4938723 T > C polymorphism was significant in subgroups, regardless of age, gender, and clinical stages. Concerning sites of origin, this polymorphism tended to reduce the risk of tumors originated from the adrenal gland but not of tumors from another site. As to the *miR-218* rs11134527 A > G polymorphism, the significant association was only observed in male subjects.

False-Positive Report Probability Results

We preset 0.2 as the false-positive report probability (FPRP) threshold. As shown in [Table 3](#), at the prior probability of 0.1, all of the significant findings for the *miR-34b/c* rs4938723 T > C polymorphism remained noteworthy, except for the results on subjects no more than 18 months old, males, and the allele contrast model. Moreover, the association with the *miR-218* rs11134527 A > G polymorphism (AG versus AA) was also noteworthy, with a statistical power of 0.820 and the FPRP value of 0.187.

DISCUSSION

In the current two-center case-control study, we investigated the association of nine polymorphisms in pre-miRNAs with neuroblastoma susceptibility in Chinese children. We found that the

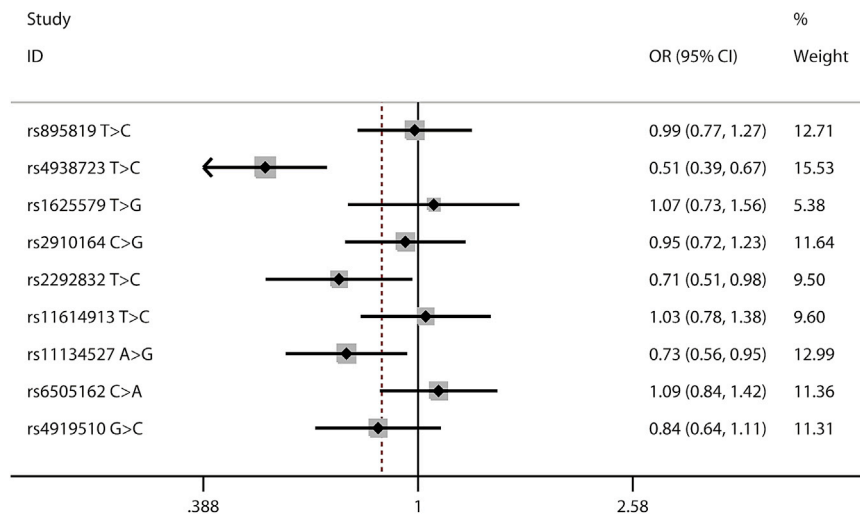


Figure 1. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Heterozygous Model: AB versus AA

For each polymorphism, the estimates of odds ratio and its 95% confidence interval are plotted with a box and a horizontal line.

This finding is consistent with those of some previous studies, such as studies conducted in cervical cancer^{43,44} and esophageal squamous cell carcinoma.⁴⁵ Opposite results were also observed. For instance, Han et al. found that the same polymorphism was associated with an increased hepatocellular carcinoma risk.⁴⁶ Polymorphisms may have diverse genetic effects on cancer susceptibility, de-

pending on different cancer types, regions, and ethnicities. It is possible that the methylation status of *miR-34b/c* may vary among different types of cancer, which could also have an impact on the risk of cancer.³⁶

This is the first and largest study to investigate the associations between polymorphisms in pre-miRNAs and neuroblastoma susceptibility in Chinese children; however, several limitations should be addressed. First, the sample size is still moderate, even though we pooled together samples from two hospitals, partially due to the low incidence rate of neuroblastoma (approximately 7.7 per million in Chinese children).⁴⁷ As a result, the statistical power of this study was relatively limited. Second, we only included nine polymorphisms in pre-miRNAs. More polymorphisms should be investigated to fully illuminate the contribution of polymorphisms in pre-miRNAs to neuroblastoma susceptibility. Third, other than polymorphisms, low-frequency coding variants and mutations undetectable by genome-wide association studies (GWASs) may also play important roles in neuroblastoma risk.⁸ More comprehensive studies are encouraged. Fourth, functional analysis is warranted to prove the biological plausibility of our findings from observational studies, which would reveal the underlying mechanisms by which the significant polymorphisms modify neuroblastoma susceptibility. Additionally, in the current hospital-based case-control study, selection bias may exist. Thus, these findings cannot be directly applied to the general population. Finally, due to the nature of retrospective studies, some demographic, environmental, and clinical characteristics were not available, which limited our ability to conduct gene-environment interactions analysis.

In conclusion, our study provides evidence that *miR-34b/c* rs4938723 T > C and *miR-218* rs11134527 A > G polymorphisms may play a protective role against neuroblastoma. Further prospective studies with different ethnicities and a large sample size are warranted to confirm our findings. In the near future, functional experiments

miR-34b/c rs4938723 T > C and *miR-218* rs11134527 A > G polymorphisms were significantly associated with a decreased neuroblastoma risk. The associations were further validated by stratified analyses and FPRP analyses. Our results indicate that the polymorphisms in pre-miRNAs may play critical roles in the etiology of neuroblastoma.

miRNAs can negatively regulate gene expressions at the posttranscriptional level and, thereby, affect cell proliferation, differentiation, apoptosis, metabolism, and carcinogenesis.²⁷ Particularly, miR-34 family members can serve as direct transcriptional targets of TP53. Loss of function of miR-34 impairs TP53-mediated cell death, while overexpression of miR-34 induces apoptosis.^{28–30} *miR-34b/c* has been reported to target TP53 and cooperate to suppress cell proliferation and adhesion-independent growth. Furthermore, TP53 can bind to the promoter region of *miR-34b/c* to increase the expression of *miR-34b/c*.³¹ The rs4938723 C > T polymorphism is located at the promoter region of *pre-miR-34b/c* (423 bp from the transcription start site), which may alter GATA-X transcription factor binding capacity and, consequently, affect the expression of target genes related to carcinogenesis.^{32,33} In 2011, Xu et al.³³ first found that carriers of the *miR-34b/c* rs4938723 T allele had a significantly increased risk of hepatocellular carcinoma. Since then, numerous epidemiology studies have been carried out to assess the role of this polymorphism in various cancers.³⁴ So far, no study investigating the association between *miR-34b/c* rs4938723 C > T polymorphism and neuroblastoma has been reported.

In the present study, we found that the rs4938723 C > T polymorphism was associated with a significantly decreased neuroblastoma risk. The *miR-34b/c* rs4938723 C > T polymorphism has been suggested to decrease the risk of intracranial aneurysm,³⁵ colorectal cancer,³⁶ esophageal squamous cell carcinoma,^{37,38} gastric cancer,^{39,40} and childhood acute lymphoblastic leukemia.^{41,42} Moreover, we also found that the *miR-218* rs11134527 A > G polymorphism was associated with a decreased neuroblastoma risk.

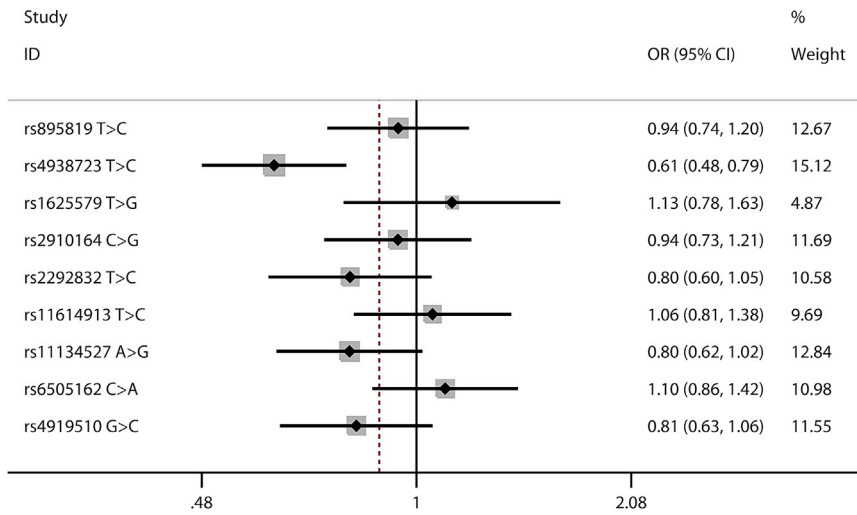


Figure 2. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Dominant Model: AB/BB versus AA

For each polymorphism, the estimates of odds ratio and its 95% confidence interval are plotted with a box and a horizontal line.

of all participants. The study protocol was approved by the institutional review boards of the participating institutions.

Polymorphism Selection and Genotyping

Nine widely investigated polymorphisms (*miR-27a* rs895819 T > C, *miR-34b/c* rs4938723 T > C, *miR-137* rs1625579 T > G, *miR-146a* rs291016 C > G, *miR-149* rs2292832 T > C,

miR-196a2 rs11614913 T > C, *miR-218* rs11134527 A > G, *miR-423* rs6505162 C > A, and *miR-608* rs4919510 G > C) were selected (Table S3). The minor allele frequency for all of the nine polymorphisms was larger than 0.05. Of them, eight were located in the transcription factor binding sites, as predicted by SNPinfo (<https://snpinfo.niehs.nih.gov/>), and the *miR-137* rs1625579 T > G polymorphism was significantly associated with schizophrenia risk.⁵³ Genomic DNA was mainly extracted from EDTA-anticoagulated blood samples by using the TIANamp Blood DNA Kit (TianGen Biotech, Beijing, China).⁵⁴ Genotyping was performed by TaqMan methodology.⁵⁵⁻⁵⁷ For quality control, 10% samples were retested, and the genotype concordance was 100%.

Statistical Analysis

The χ^2 test was used to compare the differences in the frequency distributions of demographic variables and genotypes between cases and

should be performed to explore the possible mechanisms by which these polymorphisms in pre-miRNAs modulate the development of neuroblastoma.

MATERIALS AND METHODS

Participants

The current two-center case-control study was composed of two independent retrospective studies. One study enrolled 275 histopathologically confirmed neuroblastoma cases enrolled from the Guangzhou Women and Children’s Medical Center (Guangdong Province, China), mainly between February 2010 and March 2017, and 531 cancer-free controls recruited from the same hospital as we described previously.⁴⁸⁻⁵¹ The other study incorporated 118 cases and 281 controls recruited from the First Affiliated Hospital of Zhengzhou University (Henan Province, China) from August 2011 to April 2017.⁵² Informed written consent was obtained from the guardians

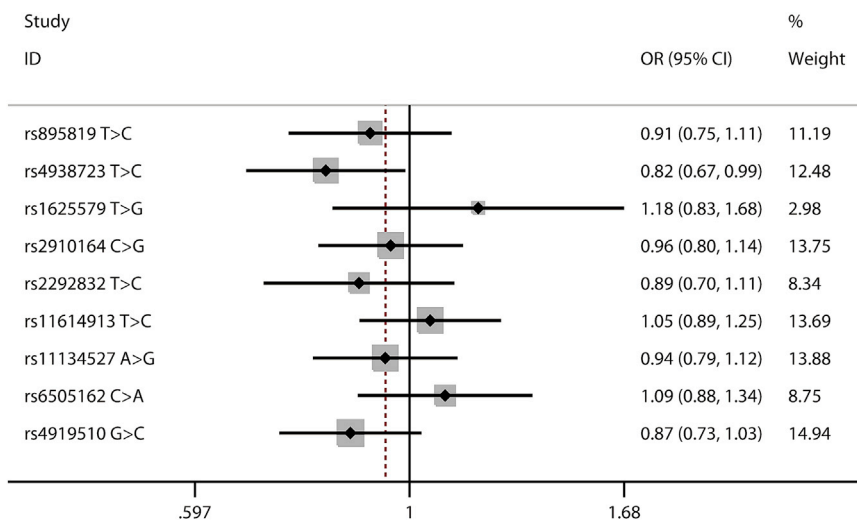


Figure 3. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Dominant Model: B versus A

For each polymorphism, the estimates of odds ratio and its 95% confidence interval are plotted with a box and a horizontal line.

Table 2. Stratified Analyses on Associations of rs4938723 T > C and rs11134527 A > G Polymorphisms with Neuroblastoma Risk

Variables	rs4938723 (Cases/ Controls)		Crude OR (95% CI)	p Value	Adjusted OR (95% CI) ^a	p Value ^a	rs11134527 (Cases/ Controls)		Crude OR (95% CI)	p Value	Adjusted OR (95% CI) ^a	p Value ^a
	TT	TC/CC					AA	AG/GG				
Age (months)												
≤ 18	75/146	45/159	0.55 (0.36–0.85) ^b	0.007 ^b	0.55 (0.36–0.85) ^b	0.007 ^b	52/107	74/198	0.77 (0.50–1.18)	0.226	0.77 (0.50–1.18)	0.227
>18	146/231	111/274	0.64 (0.47–0.87) ^b	0.004 ^b	0.64 (0.47–0.87) ^b	0.004 ^b	102/169	163/336	0.80 (0.59–1.10)	0.166	0.81 (0.59–1.10)	0.168
Gender												
Females	103/160	60/181	0.52 (0.35–0.76) ^b	0.007 ^b	0.52 (0.35–0.76) ^b	0.007 ^b	63/123	103/218	0.92 (0.63–1.35)	0.680	0.92 (0.63–1.36)	0.687
Males	118/217	96/252	0.70 (0.51–0.97) ^b	0.032 ^b	0.70 (0.51–0.97) ^b	0.031 ^b	91/153	134/316	0.71 (0.51–0.99) ^b	0.044 ^b	0.72 (0.52–0.995) ^b	0.046 ^b
Sites of Origin												
Adrenal gland	101/377	51/433	0.44 (0.31–0.63) ^b	<0.0001 ^b	0.44 (0.31–0.64) ^b	<0.0001 ^b	59/276	94/534	0.82 (0.58–1.18)	0.286	0.83 (0.58–1.19)	0.312
Retroperitoneal	35/377	42/433	1.05 (0.65–1.67)	0.855	1.05 (0.65–1.68)	0.849	32/276	54/534	0.87 (0.55–1.38)	0.561	0.86 (0.54–1.37)	0.530
Mediastinum	60/377	46/433	0.67 (0.44–1.00)	0.052	0.67 (0.45–1.02)	0.059	47/276	61/534	0.67 (0.45–1.01)	0.055	0.68 (0.45–1.02)	0.059
Other	21/377	14/433	0.58 (0.29–1.16)	0.122	0.58 (0.29–1.16)	0.121	14/276	22/534	0.81 (0.41–1.61)	0.552	0.81 (0.41–1.61)	0.547
Clinical Stages^c												
I + II + 4 s	92/377	66/433	0.63 (0.44–0.88) ^b	0.008 ^b	0.63 (0.44–0.89) ^b	0.008 ^b	65/276	97/534	0.77 (0.55–1.09)	0.141	0.78 (0.55–1.10)	0.151
III + IV	119/377	83/433	0.61 (0.44–0.83) ^b	0.002 ^b	0.60 (0.44–0.83) ^b	0.002 ^b	82/276	127/534	0.80 (0.59–1.10)	0.164	0.80 (0.59–1.10)	0.174

OR, odds ratio; CI, confidence interval; INSS, International Neuroblastoma Staging System.

^aAdjusted for age and gender, omitting the corresponding stratification factor.

^bFor these values, the 95% CI excluded 1 or p < 0.05.

^cINSS criteria defined stage 4 s as age <1 year old, with localized primary tumor as delineated in stage I or II, and with dissemination limited to liver, skin, or bone marrow.

Table 3. Results of False-Positive Report Probability Analysis for Significant Findings

Genotype and Variables	OR (95% CI)	p Value ^a	Statistical Power ^b	Prior Probability				
				0.25	0.1	0.01	0.001	0.0001
rs4938723 T > C								
TC versus TT	0.51 (0.39–0.67)	<0.0001	0.039	0.000 ^c	0.000 ^c	0.003 ^c	0.033 ^c	0.254
TC/CC versus TT	0.62 (0.48–0.79)	0.0001	0.243	0.001 ^c	0.004 ^c	0.039 ^c	0.291	0.805
C versus T	0.82 (0.68–0.99)	0.039	0.976	0.108 ^c	0.266	0.799	0.976	0.998
TC/CC versus TT								
≤18 months old	0.55 (0.36–0.85)	0.007	0.194	0.096 ^c	0.242	0.779	0.973	0.997
>18 months old	0.64 (0.47–0.87)	0.004	0.392	0.030 ^c	0.084 ^c	0.503	0.911	0.990
Females	0.52 (0.35–0.76)	0.001	0.096	0.021 ^c	0.062 ^c	0.420	0.880	0.987
Males	0.70 (0.51–0.97)	0.032	0.607	0.136 ^c	0.320	0.838	0.981	0.998
Adrenal gland as site of origin	0.44 (0.31–0.63)	<0.0001	0.015	0.002 ^c	0.006 ^c	0.062 ^c	0.399	0.869
I + II + 4 s	0.63 (0.44–0.88)	0.008	0.351	0.060 ^c	0.161 ^c	0.679	0.955	0.995
III + IV	0.61 (0.44–0.83)	0.002	0.278	0.019 ^c	0.055 ^c	0.391	0.866	0.985
rs11134527 A > G								
AG versus AA	0.73 (0.56–0.95)	0.021	0.820	0.071 ^c	0.187 ^c	0.717	0.962	0.996
Males	0.71 (0.51–0.99)	0.044	0.648	0.169 ^c	0.378	0.870	0.985	0.999

OR, odds ratio; CI, confidence interval.

^aChi-square test was used to calculate the genotype frequency distributions.

^bStatistical power was calculated using the number of observations in each subgroup and the corresponding ORs and p values in this table.

^cThe level of false-positive report probability threshold was set at 0.2 and noteworthy findings are presented.

controls. The goodness-of-fit χ^2 test was adopted to evaluate departure from HWE for the selected polymorphisms in control subjects. ORs and 95% CIs, calculated by multivariate logistic regression, were used to assess the association between the nine selected polymorphisms and neuroblastoma susceptibility. Additionally, stratified analyses were performed by age, gender, tumor sites, and clinical stages. Moreover, we also performed FPRP analysis to verify significant results from the combined subjects.^{16,58} All data were analyzed using SAS software (v9.4; SAS Institute, Cary, NC, USA). The p values less than 0.05 were considered as statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four tables and can be found with this article online at <https://doi.org/10.1016/j.omtn.2018.01.003>.

AUTHOR CONTRIBUTIONS

Participated in research design: J.H., and H.X.; Conducted experiments: J.H., X.L., and R.Z.; Collected samples: J.H., Y.Z., J. Zhang, and T.Y.; Performed data analysis: J.H. and J. Zhu. Wrote or contributed to the writing of the manuscript: J.H., J. Zhu, and H.X.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Supplemental Information

**Association of Common Genetic Variants in
Pre-microRNAs and Neuroblastoma Susceptibility:
A Two-Center Study in Chinese Children**

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Table S1 Frequency distribution of selected characteristics in neuroblastoma cases and cancer-free controls

Variables	Guangdong province				<i>P</i> ^a	Henan province				<i>P</i> ^a
	Cases (N=275)		Controls (N=531)			Cases (N=118)		Controls (N=281)		
	No.	%	No.	%		No.	%	No.	%	
Age range, month	0-132		0.07-156		0.229	0-131.1		0.1-144.0		0.484
Mean ± SD	31.50±25.43		29.73±24.86			46.24±29.98		44.97±33.23		
<12	70	25.45	145	27.31		9	7.63	32	11.39	
12-60	177	64.36	313	58.95		76	64.41	179	63.70	
>60	28	10.18	73	13.75		33	27.97	70	24.91	
Gender					0.510					0.196
Female	114	41.45	233	43.88		54	45.76	109	38.79	
Male	161	58.55	298	56.12		64	54.24	172	61.21	
Clinical stages ^b										
I	54	19.64				15	12.71			
II	62	22.55				31	26.27			
III	49	17.82				19	16.10			
IV	94	34.18				49	41.53			
4s	8	2.91				3	2.54			
NA	8	2.91				1	0.85			
Sites of origin										
Adrenal gland	64	23.27				89	75.42			
Retroperitoneal region	87	31.64				/	/			
Mediastinum	90	32.73				19	16.10			
Other region	26	9.45				10	8.47			
NA	8	2.91				/	/			

SD, standard deviation; NA, not available.

^a Two-sided χ^2 test for distributions between neuroblastoma cases and cancer-free controls.

^b INSS criteria defined stage 4s as age <1 year old with localized primary tumor as delineated in stage I or II, with dissemination limited to liver, skin, or bone marrow.

Table S2 Logistic regression analyses on associations between selected polymorphisms and neuroblastoma risk in Chinese children (homozygous and allele comparing models)

miRNA	SNP	Allele		Case (N=393)			Control (N=812)			Homozygous (BB vs. AA)		Allele comparing (B vs. A)	
		A	B	AA	AB	BB	AA	AB	BB	AOR ^a (95% CI)	P ^a	AOR ^a (95% CI)	P ^a
<i>miR-27a</i>	rs895819	T	C	220	153	20	442	312	58	0.70 (0.41-1.19)	0.185	0.91 (0.75-1.11)	0.354
<i>miR-34b/c</i>	rs4938723	T	C	221	107	49	377	358	75	1.11 (0.75-1.66)	0.597	0.82 (0.68-0.99)	0.039
<i>miR-137</i>	rs1625579	T	G	343	46	3	719	90	1	6.28 (0.65-60.51)	0.112	1.18 (0.83-1.68)	0.354
<i>miR-146a</i>	rs2910164	C	G	142	189	60	282	397	130	0.91 (0.63-1.32)	0.621	0.95 (0.80-1.14)	0.591
<i>miR-149</i>	rs2292832	T	C	286	62	32	560	172	59	1.06 (0.68-1.67)	0.789	0.88 (0.70-1.11)	0.294
<i>miR-196a2</i>	rs11614913	T	C	107	192	94	230	399	183	1.10 (0.79-1.55)	0.566	1.05 (0.89-1.25)	0.566
<i>miR-218</i>	rs11134527	A	G	154	164	73	276	403	131	1.00 (0.70-1.41)	0.986	0.94 (0.79-1.12)	0.504
<i>miR-423</i>	rs6505162	C	A	244	132	17	522	258	31	1.16 (0.63-2.15)	0.626	1.08 (0.88-1.34)	0.436
<i>miR-608</i>	rs4919510	G	C	127	190	76	227	405	179	0.76 (0.54-1.08)	0.112	0.87 (0.73-1.03)	0.099

AOR, adjusted odds ratio; CI, confidence interval.

The results were in bold if the 95% CI excluded 1 or $P < 0.05$.

^a Adjusted for age and gender.

Table S3 Function of selected polymorphisms as predicted by SNPinfo (<http://snpinfo.niehs.nih.gov/>) software

miRNA	rs	Chr	Allele	TFBS	Splicing (ESE or ESS)	Allele	Asian	CHB
<i>miR-27a</i>	rs895819	19	C/T	Y	Y	T	--	0.711
<i>miR-34b/c</i>	rs4938723	11	C/T	Y	--	T	0.669	0.643
<i>miR-137</i>	rs1625579	1	T/G	--	--	T	0.947	0.940
<i>miR-146a</i>	rs2910164	5	C/G	Y	Y	G	0.469	0.446
<i>miR-149</i>	rs2292832	2	C/T	Y	Y	C	0.339	0.315
<i>miR-196a2</i>	rs11614913	12	C/T	Y	Y	C	0.427	0.411
<i>miR-218</i>	rs11134527	5	A/G	Y	--	G	0.417	0.367
<i>miR-423</i>	rs6505162	17	A/C	Y	Y	C	0.811	0.833
<i>miR-608</i>	rs4919510	10	C/G	Y	Y	C	0.491	0.488

TFBS, transcription factor binding sites; ESE, exon splicing enhancer; ESS, exon splicing silencer; CHB, Han Chinese in Beijing, China.

Table S4 Association of *miR-34b/c* rs4938723 T>C and *miR-218* rs11134527 A>G polymorphisms with neuroblastoma susceptibility

Genotype	Guangdong province				Henan province				Combined			
	Cases (N=275)	Controls (N=531)	Adjusted OR (95% CI) ^a	P ^a	Cases (N=118)	Controls (N=281)	Adjusted OR (95% CI) ^a	P ^a	Cases (N=393)	Controls (N=812)	Adjusted OR (95% CI) ^a	P ^a
rs4938723 T>C												
TT	147 (56.76)	244 (46.12)	1.00		74 (62.71)	133 (47.33)	1.00		221 (58.62)	377 (46.54)	1.00	
TC	80 (30.89)	236 (44.61)	0.56 (0.41-0.78)	0.0005	27 (22.88)	122 (43.42)	0.40 (0.24-0.67)	0.0004	107 (28.38)	358 (44.20)	0.51 (0.39-0.67)	<0.0001
CC	32 (12.36)	49 (9.26)	1.07 (0.65-1.75)	0.797	17 (14.41)	26 (9.25)	1.15 (0.58-2.26)	0.690	49 (13.00)	75 (9.26)	1.11 (0.75-1.66)	0.597
Additive			0.84 (0.67-1.05)	0.122			0.79 (0.57-1.10)	0.162			0.83 (0.69-0.997)	0.046
Dominant	112 (43.24)	285 (53.88)	0.65 (0.48-0.88)	0.005	44 (37.29)	148 (52.67)	0.54 (0.35-0.83)	0.006	156 (41.38)	433 (53.46)	0.62 (0.48-0.79)	0.0001
Recessive	227 (87.64)	480 (90.74)	1.36 (0.85-2.19)	0.202	101 (85.59)	255 (90.75)	1.60 (0.83-3.08)	0.162	328 (87.00)	735 (90.74)	1.46 (1.00-2.15)	0.052
T	374 (72.20)	724 (68.43)	1.00		175 (74.15)	388 (69.04)	1.00		549 (72.81)	1112 (68.64)	1.00	
C	144 (27.80)	334 (31.57)	0.83 (0.66-1.05)	0.113	61 (25.85)	174 (30.96)	0.77 (0.55-1.09)	0.141	205 (27.19)	508 (31.36)	0.82 (0.68-0.99)	0.039
rs11134527 A>G												
AA	107 (39.19)	162 (30.62)	1.00		47 (39.83)	114 (40.57)	1.00		154 (39.39)	276 (34.07)	1.00	
AG	117 (42.86)	286 (54.06)	0.62 (0.45-0.86)	0.004	47 (39.83)	117 (41.64)	0.98 (0.61-1.59)	0.940	164 (41.94)	403 (49.75)	0.73 (0.56-0.96)	0.022
GG	49 (17.95)	81 (15.31)	0.90 (0.58-1.38)	0.618	24 (20.34)	50 (17.79)	1.17 (0.65-2.12)	0.602	73 (18.67)	131 (16.17)	1.00 (0.70-1.41)	0.986
Additive			0.87 (0.70-1.08)	0.211			1.07 (0.80-1.43)	0.666			0.94 (0.79-1.12)	0.509
Dominant	166 (60.81)	367 (69.38)	0.68 (0.50-0.93)	0.014	71 (60.17)	167 (59.43)	1.04 (0.67-1.61)	0.866	237 (60.61)	534 (65.93)	0.80 (0.62-1.02)	0.073
Recessive	224 (82.05)	448 (84.69)	1.18 (0.80-1.75)	0.401	94 (79.66)	231 (82.21)	1.18 (0.69-2.04)	0.546	318 (81.33)	679 (83.83)	1.19 (0.86-1.63)	0.290
A	331 (60.62)	610 (57.66)	1.00		141 (59.75)	345 (61.39)	1.00		472 (60.36)	955 (58.95)	1.00	
G	215 (39.38)	448 (42.34)	0.88 (0.71-1.08)	0.219	95 (40.25)	217 (38.61)	1.08 (0.79-1.47)	0.645	310 (39.64)	665 (41.05)	0.94 (0.79-1.12)	0.504

OR, odds ratio; CI, confidence interval.

The results were in bold if the 95% CI excluded 1 or $P < 0.05$.^a χ^2 test for genotype distributions between neuroblastoma patients and controls.^b Adjusted for age and gender.