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# 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-association-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 | 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] | |
| *miR-27a* | 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 |
| *miR-34b/c* | 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 |
| *miR-137* | 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 |
| *miR-146a* | 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 |
| *miR-149* | 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 |
| *miR-196a2* | 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 |
| *miR-218* | 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 |
| *miR-423* | 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 |
| *miR-608* | 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.
[a]Adjusted for age and gender.
[b]For 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

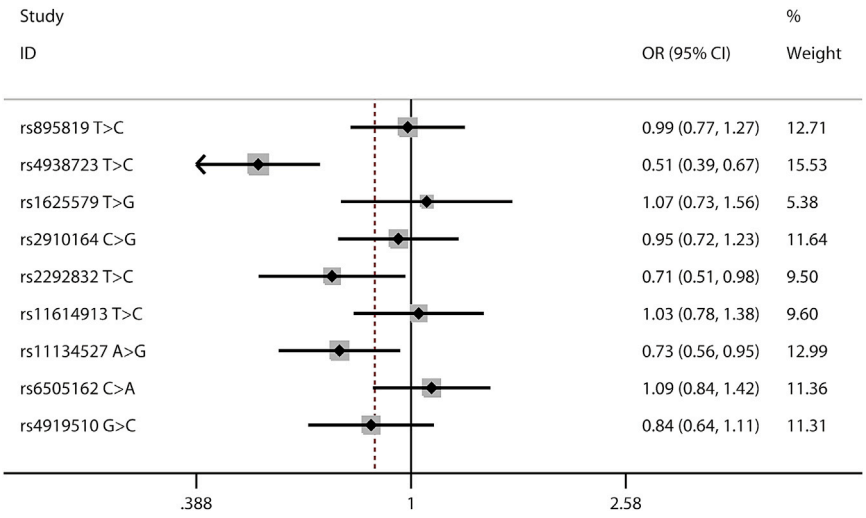


**Figure 1. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Heterozygous Model: AB versus AA**

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

*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 *pri-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. 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, depending 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-environmental 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

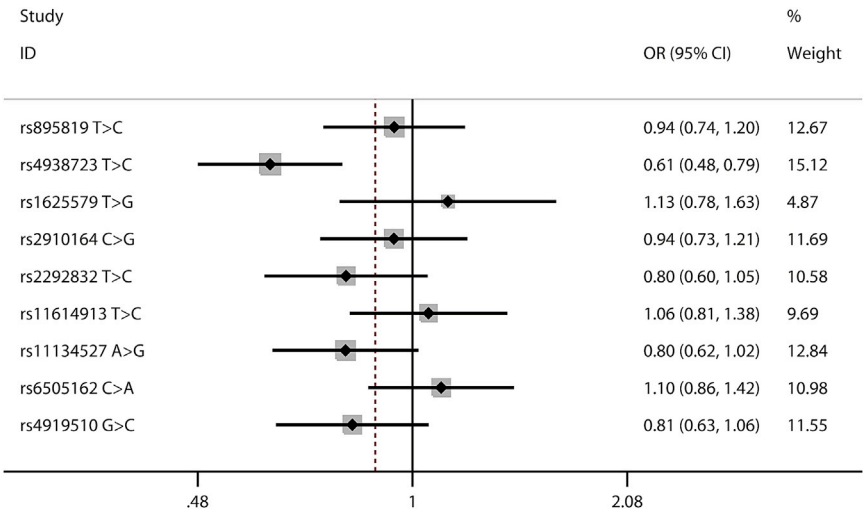


**Figure 2. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Dominant Model: AB/BB versus AA**

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

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 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 $\chi^2$ test was used to compare the differences in the frequency distributions of demographic variables and genotypes between cases and

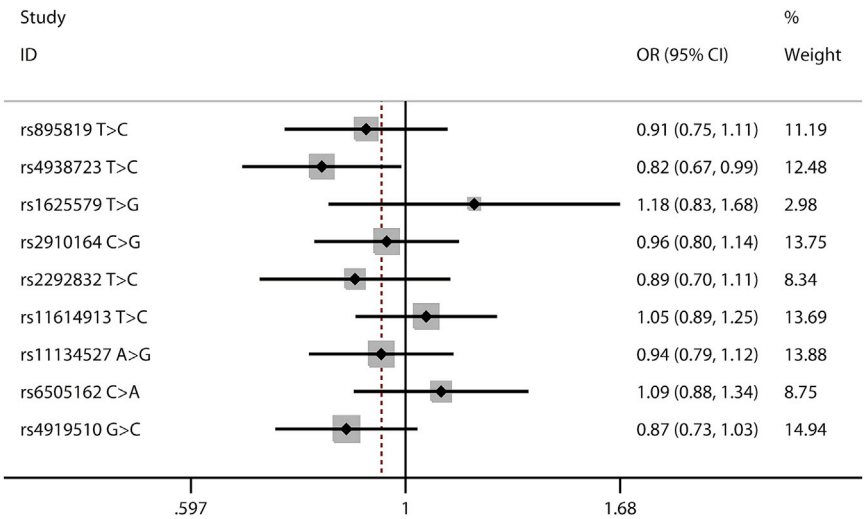


**Figure 3. Forest Plot for Association between Selected Polymorphisms and Neuroblastoma Susceptibility by a Dominant Model: B versus A**

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**

| | rs4938723 (Cases/Controls) | | | | | | rs11134527 (Cases/Controls) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | TT | TC/CC | Crude OR (95% CI) | p Value | Adjusted OR (95% CI)[a] | p Value[a] | AA | AG/GG | Crude OR (95% CI) | p Value | Adjusted OR (95% CI)[a] | p Value[a] |
| 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.

[a]Adjusted for age and gender, omitting the corresponding stratification factor.

[b]For these values, the 95% CI excluded 1 or $p < 0.05$.

[c]INSS 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.
[a]Chi-square test was used to calculate the genotype frequency distributions.
[b]Statistical power was calculated using the number of observations in each subgroup and the corresponding ORs and p values in this table.
[c]The level of false-positive report probability threshold was set at 0.2 and noteworthy findings are presented.

controls. The goodness-of-fit $\chi^2$ 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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