Research Article

Associations between H19 polymorphisms and neuroblastoma risk in Chinese children

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Background
H19 polymorphisms have been reported to correlate with an increased susceptibility to a few types of cancers, although their role in neuroblastoma has not yet been clarified.

Materials and methods
We investigated the association between three single polymorphisms (rs2839698 G>A, rs3024270 C>G, and rs217727 G>A) and neuroblastoma susceptibility in Chinese Han populations. Three hundred ninety-three neuroblastoma patients and 812 healthy controls were enrolled from the Henan and Guangdong provinces. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the strength of the association of interest.

Results
Separated and combined analyses revealed no associations of the rs2839698 G>A, rs3024270 C>G or rs217727 G>A polymorphisms and neuroblastoma susceptibility. In the stratification analysis, female children with rs3024270 GG genotypes had an increased neuroblastoma risk (adjusted OR = 1.61, 95% CI = 1.04–2.50, P = 0.032).

Conclusion
The rs3024270 G>G genotype might contribute to an increased neuroblastoma susceptibility in female Chinese children.

Background
Neuroblastoma is the most common malignant extracranial solid tumor in children, accounting for 7–10% of all tumors in children [1,2]. Neuroblastoma originates from neural crest precursor cells of the sympathetic nervous system and is mainly located on the adrenal medulla, paraspinal ganglia, and sympathetic trunk [3–5]. The outcome of neuroblastoma is affected by several factors, such as age of onset, pathology subtypes, International Neuroblastoma Staging System (INSS) stage, c-Myc status, DNA ploidy, and structural chromosomal aberrations [3–5].

Genetic factors are critically important in neuroblastoma tumorigenesis. Approximately 1% of patients have a family history of neuroblastoma, and these patients are carriers of certain gene mutations. For example, the anaplastic lymphoma kinase (ALK) gene and paired-like homeobox 2b (PHOX2B) gene have been identified as predisposing factors to familial neuroblastoma [6–8]. Evidence from genome-wide association studies (GWASs) of sporadic cases also suggests that genetic factors might be involved in the pathogenesis of neuroblastoma [9,10]. These studies indicate an important role of genetic characteristics in the tumorigenesis of neuroblastoma. Single nucleotide polymorphisms (SNPs) or other gene mutations, such as rare mutations in the ALK gene at locus 2p23 for familial neuroblastoma, common SNPs in cancer susceptibility candidate 15 (CASC15) and neuroblastoma-associated transcript 1 (NBAT1) at 6p22 were found to be involved in tumor initiation of neuroblastoma, and the bos taurus BRCA1 associated RING domain 1 (BARD1) gene at 2q35, IL3, cholesterol-lowering factor (CFL), and lens intrinsic membrane (LIM) domain only 1 (LMO1) were found to be related to the risk of sporadic neuroblastoma.

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H19 is a paternally imprinted gene that is located at chromosome 11p15.5 near the IGF2 gene. The expression of H19 peaks during embryogenesis and is down-regulated after birth in most tissues. Increasing evidence has revealed that H19 plays a critical role during tumorigenesis. H19 has been found to re-express during tumorigenesis in many kinds of tumors, such as breast cancer [11], lung cancer [12], invasive cervical carcinomas [13], esophageal cancer [14], and bladder cancer [15]. Smoking, exposure to the carcinogens, nitrosamine and diethylnitrosamine, and hypoxia are risk factors for H19 overexpression [16]. The up-regulation of H19 enhances resistance of cancer cells to stress, such as genome destabilization and hypoxia [17].

Recently, a GWAS revealed that SNPs in H19 are associated with cancer susceptibility [18], and the correlation of H19 polymorphisms and cancers were confirmed in subsequent studies [19–21]. A study on colorectal cancer reported that the SNPs rs2839698 G>A, rs3024270 C>G, and rs217727 G>A, which are located at exon 1, exon 5, and intron region of the H19 gene, respectively, have effects on the secondary structure of H19 [20], and these SNPs were found to be associated with an increased cancer risk in Chinese populations [20,22–27]. However, few studies have focused on neuroblastoma. Herein, we performed a two-center hospital-based case–control study using data from 393 neuroblastoma patients and 812 control subjects to evaluate the association between the H19 gene rs2839698, rs3024270, and rs217727 polymorphisms and neuroblastoma risk in Chinese children.

Materials and methods
Study subjects
The subjects enrolled were described in previous studies [28,29]. Briefly, a total of 393 neuroblastoma patients and 812 cancer-free controls from two provinces of China were enrolled in the present study. Two hundred seventy-five neuroblastoma patients and 531 controls were recruited from the Guangzhou Women and Children’s Medical Center in South China, and 118 neuroblastoma patients and 281 controls were recruited from the Henan province in North China [30,31]. The diagnosis and clinical stages of neuroblastoma were made according to the INSS [32]. The healthy controls had no history of malignancies and were matched to the neuroblastoma cases in terms of age (±5 years), sex, ethnicity, and geographical region [33–35]. Both neuroblastoma patients and controls were unrelated Chinese Han individuals living in the Guangdong and Henan provinces of China. The present study was approved by the Institutional Review Board of each hospital, and written informed consent was obtained from the participants’ parents or legal guardians.

SNP selection and genotyping
The dbSNP database and SNPinfo were used for selecting candidate SNPs. Three widely investigated SNPs (rs2839698, rs3024270, and rs217727) in the H19 gene were studied. SNP genotyping was performed by the TaqMan real-time PCR system as previously reported [36–40]. To validate the accuracy of the genotyping results from TaqMan real-time PCR, approximately 10% of the samples were randomly selected as quality control samples and genotyped using sequencing. The concordance for the quality control samples was 100%.

Statistical analysis
All statistical tests were two-sided, with a significance level of $P<0.05$. All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, U.S.A.). Two-sided $\chi^2$ tests were used to analyze the demographic data and genotype frequencies. The Hardy–Weinberg equilibrium was assessed by goodness-of-fit $\chi^2$ test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate associations between H19 polymorphisms and neuroblastoma susceptibility.

Results
Associations between H19 gene polymorphisms and neuroblastoma susceptibility
In the present study, 392 patients and 810 controls were successfully genotyped. The genotype frequencies of all the three selected SNPs were in Hardy–Weinberg equilibrium (rs2839698, $P=0.090$; rs3024270, $P=0.159$; rs217727, $P=0.470$). The genotype frequencies of the three SNPs in the patients and controls are listed in Table 1. None of the three SNPs were found to be associated with an increased neuroblastoma risk in the separate analyses of each SNP or in the combined analysis on protective genotypes of the three SNPs.
Table 1 Logistic regression analysis for the associations between H19 polymorphisms and neuroblastoma risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n=393)</th>
<th>Controls (n=810)</th>
<th>P1</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)2</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2839698</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>179 (45.55)</td>
<td>365 (45.06)</td>
<td></td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>175 (44.53)</td>
<td>373 (46.05)</td>
<td></td>
<td>0.96 (0.74–1.23)</td>
<td>0.732</td>
<td>0.96 (0.75–1.24)</td>
<td>0.754</td>
</tr>
<tr>
<td>AA</td>
<td>39 (9.92)</td>
<td>72 (8.89)</td>
<td></td>
<td>1.11 (0.72–1.70)</td>
<td>0.650</td>
<td>1.11 (0.73–1.71)</td>
<td>0.624</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
<td>0.797</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>214 (54.45)</td>
<td>445 (54.94)</td>
<td></td>
<td>1.01 (0.84–1.22)</td>
<td>0.890</td>
<td>1.02 (0.84–1.23)</td>
<td>0.858</td>
</tr>
<tr>
<td>Recessive</td>
<td>354 (90.08)</td>
<td>738 (91.11)</td>
<td></td>
<td>1.13 (0.75–1.70)</td>
<td>0.561</td>
<td>1.14 (0.75–1.71)</td>
<td>0.541</td>
</tr>
<tr>
<td>rs3024270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>99 (25.19)</td>
<td>213 (26.30)</td>
<td></td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>203 (51.65)</td>
<td>424 (52.35)</td>
<td></td>
<td>1.03 (0.77–1.38)</td>
<td>0.842</td>
<td>1.03 (0.77–1.38)</td>
<td>0.829</td>
</tr>
<tr>
<td>GG</td>
<td>91 (23.16)</td>
<td>173 (21.36)</td>
<td></td>
<td>1.13 (0.80–1.60)</td>
<td>0.486</td>
<td>1.14 (0.80–1.61)</td>
<td>0.472</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
<td>0.764</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>294 (74.81)</td>
<td>597 (73.70)</td>
<td></td>
<td>1.06 (0.89–1.27)</td>
<td>0.494</td>
<td>1.07 (0.89–1.27)</td>
<td>0.480</td>
</tr>
<tr>
<td>Recessive</td>
<td>302 (76.84)</td>
<td>637 (78.64)</td>
<td></td>
<td>1.11 (0.83–1.48)</td>
<td>0.480</td>
<td>1.11 (0.83–1.48)</td>
<td>0.470</td>
</tr>
<tr>
<td>rs217727</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>186 (47.33)</td>
<td>382 (47.16)</td>
<td></td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>164 (41.73)</td>
<td>342 (42.22)</td>
<td></td>
<td>0.99 (0.76–1.27)</td>
<td>0.907</td>
<td>0.98 (0.76–1.27)</td>
<td>0.888</td>
</tr>
<tr>
<td>AA</td>
<td>43 (10.94)</td>
<td>86 (10.62)</td>
<td></td>
<td>1.03 (0.68–1.54)</td>
<td>0.898</td>
<td>1.02 (0.68–1.53)</td>
<td>0.922</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
<td>0.979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>207 (52.67)</td>
<td>428 (52.84)</td>
<td></td>
<td>1.00 (0.84–1.20)</td>
<td>0.970</td>
<td>1.00 (0.84–1.20)</td>
<td>0.997</td>
</tr>
<tr>
<td>Recessive</td>
<td>350 (89.06)</td>
<td>724 (89.38)</td>
<td></td>
<td>0.99 (0.78–1.26)</td>
<td>0.956</td>
<td>0.99 (0.78–1.26)</td>
<td>0.932</td>
</tr>
<tr>
<td>Combined effect of protective genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>258 (65.65)</td>
<td>551 (68.02)</td>
<td></td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>135 (34.35)</td>
<td>259 (31.98)</td>
<td></td>
<td>1.11 (0.86–1.44)</td>
<td>0.410</td>
<td>1.11 (0.86–1.44)</td>
<td>0.411</td>
</tr>
</tbody>
</table>

1 χ² test for genotype distributions between neuroblastoma patients and cancer-free controls.
2 Adjusted for age and sex.

Stratification analysis

We next analyzed the association of the three SNPs with neuroblastoma risk by stratification analyses (Table 2). Stratification analyses were conducted based on age (≤18 months, >18 months), sex (female, male), site of origin (adrenal gland, retroperitoneal, mediastinum, other sites), and clinical stage (I+II+4s, III+IV). We found that the increased risk associated with the rs3024270 GG variant genotype was more pronounced in females (adjusted OR = 1.61, 95% CI = 1.04–2.50, P=0.032). Regarding the risk genotypes, no significant associations between the 1–3 risk genotypes and neuroblastoma risk were found.

Discussion

In the present study, we found the H19 gene rs3024270 C>G polymorphism was associated with neuroblastoma susceptibility in females. This is the first study to report an association between the rs3024270 GG genotype and an increased neuroblastoma risk in female Chinese children in a recessive model.

The association between these SNPs and cancer risk has been studied, while evidence of rs3024270 GG genotype and cancer risk are relatively limited. A study by Li et al. [20] reported an increased risk of colorectal cancer in a Chinese population with the rs3024270 GG genotype. A meta-analysis conducted by Lv et al. [21] investigated the association between long noncoding RNA polymorphisms and cancer risk, and the rs3024270 C>G polymorphism was reported to be correlated with an overall cancer risk. However, another study found a decreased risk of invasive bladder cancer in patients with the rs3024270 CC genotype in the Chinese population [41]. Besides the rs3024270 C>G polymorphism, a series of studies reported the role of the rs2839698 G>A polymorphism in platinum-based chemotherapy response in lung cancer [42], colorectal cancer [20], gastric cancer [24] and rheumatoid arthritis [22]. The rs217727 G>A polymorphism was also reported to be associated with a risk of oral squamous cell carcinoma [19], osteosarcoma [43], bladder cancer [44], cervical cancer of the squamous carcinomas subtype [20], and breast cancer [27]. Our study provides new evidence that the rs3024270 C>G polymorphism in the H19 gene might be involved in the development of neuroblastoma.
Table 2 Stratification analysis for association between \textit{H19} genotypes and neuroblastoma susceptibility

<table>
<thead>
<tr>
<th>Variables</th>
<th>rs2839698 (patient/control)</th>
<th>Adjusted OR$^1$</th>
<th>P$^1$</th>
<th>rs3024270 (patient/control)</th>
<th>Adjusted OR$^1$</th>
<th>P$^1$</th>
<th>rs217727 (patient/control)</th>
<th>Adjusted OR$^1$</th>
<th>P$^1$</th>
<th>Risk genotypes (patient/control)</th>
<th>Adjusted OR$^1$</th>
<th>P$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months</td>
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</tr>
<tr>
<td>≤ 18</td>
<td>110/276</td>
<td>15/29</td>
<td>1.29 (0.66–2.50)</td>
<td>0.452</td>
<td>95/240</td>
<td>31/66</td>
<td>1.21 (0.74–1.97)</td>
<td>0.454</td>
<td>1.21 (0.75–1.52)</td>
<td>1.16/270</td>
<td>10/35</td>
<td>0.67 (0.32–1.39)</td>
</tr>
<tr>
<td>&gt; 18</td>
<td>243/462</td>
<td>24/43</td>
<td>1.06 (0.63–1.79)</td>
<td>0.630</td>
<td>207/397</td>
<td>60/108</td>
<td>1.07 (0.75–1.52)</td>
<td>0.727</td>
<td>1.25 (0.79–2.00)</td>
<td>234/454</td>
<td>33/51</td>
<td>0.342</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>148/317</td>
<td>20/24</td>
<td>1.79 (0.96–3.34)</td>
<td>0.068</td>
<td>123/278</td>
<td>45/63</td>
<td>1.61 (1.04–2.50)</td>
<td>0.032</td>
<td>1.07 (0.45–1.53)</td>
<td>152/302</td>
<td>16/39</td>
<td>0.83 (0.45–1.53)</td>
</tr>
<tr>
<td>Male</td>
<td>206/421</td>
<td>19/48</td>
<td>0.83 (0.48–1.45)</td>
<td>0.516</td>
<td>179/359</td>
<td>46/110</td>
<td>0.85 (0.57–1.25)</td>
<td>0.398</td>
<td>1.22 (0.74–2.02)</td>
<td>198/422</td>
<td>27/47</td>
<td>0.442</td>
</tr>
<tr>
<td>Sites of origin</td>
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<td></td>
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</tr>
<tr>
<td>Adrenal gland</td>
<td>140/738</td>
<td>13/72</td>
<td>0.97 (0.52–1.80)</td>
<td>0.915</td>
<td>115/637</td>
<td>38/173</td>
<td>1.23 (0.82–1.84)</td>
<td>0.327</td>
<td>1.24 (0.73–2.08)</td>
<td>133/724</td>
<td>20/86</td>
<td>0.430</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>79/738</td>
<td>8/72</td>
<td>1.00 (0.47–2.18)</td>
<td>0.982</td>
<td>67/637</td>
<td>20/173</td>
<td>1.06 (0.64–1.83)</td>
<td>0.776</td>
<td>0.52 (0.21–1.32)</td>
<td>82/724</td>
<td>5/86</td>
<td>0.169</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>98/738</td>
<td>11/72</td>
<td>1.16 (0.59–2.27)</td>
<td>0.661</td>
<td>89/637</td>
<td>20/173</td>
<td>0.84 (0.50–1.40)</td>
<td>0.493</td>
<td>1.25 (0.68–2.28)</td>
<td>95/724</td>
<td>14/86</td>
<td>0.478</td>
</tr>
<tr>
<td>Others</td>
<td>30/738</td>
<td>6/72</td>
<td>2.00 (0.81–4.99)</td>
<td>0.135</td>
<td>24/637</td>
<td>12/173</td>
<td>1.82 (0.89–3.73)</td>
<td>0.100</td>
<td>0.50 (0.12–2.13)</td>
<td>34/724</td>
<td>2/86</td>
<td>0.350</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II+IVs</td>
<td>146/738</td>
<td>16/72</td>
<td>1.13 (0.64–2.00)</td>
<td>0.677</td>
<td>126/637</td>
<td>36/173</td>
<td>1.06 (0.70–1.59)</td>
<td>0.792</td>
<td>1.13 (0.66–1.91)</td>
<td>143/724</td>
<td>19/86</td>
<td>0.661</td>
</tr>
<tr>
<td>III+IV</td>
<td>190/738</td>
<td>21/72</td>
<td>1.17 (0.70–1.95)</td>
<td>0.561</td>
<td>160/637</td>
<td>51/173</td>
<td>1.19 (0.83–1.71)</td>
<td>0.335</td>
<td>1.06 (0.65–1.71)</td>
<td>187/724</td>
<td>24/86</td>
<td>0.821</td>
</tr>
</tbody>
</table>

$^1$Adjusted for age and sex.
Values in bold indicate $P<0.05$.系
SNPs studied in our research are all located in the H19 gene and were reported to have an impact on the folding structure of H19 [20], which may affect the expression or functions of H19. The role of H19 in cancers has been widely studied. The H19 gene is an imprinted oncofetal gene that is located on chromosome 11p15.5 near the IGF2 gene locus [45]. Wada et al. [46] reported a maintenance of normal imprinting of the H19 and IGF2 genes in neuroblastoma. The expression of H19 was significantly up-regulated in hepatic metastases of several types of cancers [47]. Growing evidence indicates that H19 is involved in both the proliferation and differentiation processes of cancer cells. H19 promotes cell cycle progression in breast cancer, while the depletion of H19 RNA arrests breast cancer cells in the pre-S-phase of the cell cycle [48]. The knockdown of H19 inhibits the growth of HCC and gastric cancer cells under hypoxic recovery conditions [49].

The mechanisms by which these SNPs regulate H19 expression and are involved in cancer are unclear. A possible explanation is that SNPs may affect H19 gene expression and function. It was reported that rs3024270 C>G, rs2839698 G>A, and rs217727 G>A dramatically altered the secondary structure of H19, and the rs3024270 GG genotype was associated with an increased risk of colorectal cancer in a Chinese population. H19 was found to act as a primary microRNA precursor, and H19 expression regulated specific miRNAs via post transcription [50]. Liang et al. [54] reported that IncRNA H19 modulated the expression of multiple genes involved in colorectal cancer by acting as a competing endogenous RNA, and H19 was also reported to produce antisense 91H, which increased the stability in cancer cells, leading to the accumulation of 91H in cancer cell lines and breast cancer tissues [55]. SNPs located in the introns and exons of the H19 gene might change the promoter activity and function of H19 by the alteration of target miRNAs, such as hsa-miR-24-1-5p, hsa-miR-4486, and hsa-miR-566 [55]. Additionally, H19 was also found to interact with other tumor suppressor genes, such as p53 and c-Myc. Increased expression of H19 during hypoxic conditions was found in cell lines with a mutant p53 gene but not in cells with wild-type p53 gene [50], and overexpression of H19 partially suppressed p53 activation in gastric cancer cells [51]. H19 expression was activated by c-Myc via direct binding to the regulatory region of the H19 gene [52].

In our study, we found the GG allele pattern in 45/63 female NB patients and in 46/110 male NB patients. In the stratification analysis based on sex, disequilibrium of the G allele and the C allele was found. H19 is an imprinted gene that is expressed from the maternal chromosome, but not the paternal chromosome [45]. This fact might partly explain the apparent disequilibrium of the analyzed SNPs. In the stratified analysis, no relation of H19 SNPs with the susceptibility of neuroblastoma patients, the origin of the tumor and the clinical stage of the tumor were found. In other studies, no relationship between rs3024270 and the susceptibility of cancer was found in stratified analyses, including age, sex, smoking, drinking etc. Further studies with larger sample sizes of patients with neuroblastoma and other tumors may provide more information.

There are several limitations of this case–control study. First, a potential selection bias would be induced because the enrollment of cases and controls occurred in a hospital. Therefore, further studies with larger populations would be of great value. Second, only three SNPs in the H19 were included in the current study, and other polymorphisms, such as rs2735971 C>T, were not included. Furthermore, the biological functions of the three SNPs are not clear, so functional studies are needed to provide more evidence. The sample size is relatively small in the present study due to the difficulty in sample collection. We will continue our study on neuroblastoma and update the results in further study.

Conclusion
In the present study, we reported the relationship between the rs3024270 GG genotype and the increased susceptibility of neuroblastoma in female Chinese children. Further functional studies on rs3024270 and studies with larger populations and different ethnicities would be valuable to clarify the role of H19 SNPs in neuroblastoma.

Author contribution
C.H. and T.Y. made substantial contributions to conception and design of the present study. J.P., J.Z., and J.Y. made substantial contribution to the acquisition and interpretation of data. C.H. made substantial contribution to statistical analysis and interpretation of data. J.H. and Y.Z. had been involved in drafting the manuscript and revising it critically for important intellectual content. Y.Z. and J.H. agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. C.H. and T.Y. contributed to the work equally. All authors had given final approval of the version to be published.

Data availability
The data used to support the findings of this study are available from the corresponding authors upon request.

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Competing interests
The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
ALK, anaplastic lymphoma kinase; CI, confidence interval; GWAS, genome-wide association study; INSS, International Neuroblastoma Staging System; OR, odds ratio; SNP, single nucleotide polymorphism.

References

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