Research Article

Association of miR-146a, miR-149 and miR-196a2 polymorphisms with neuroblastoma risk in Eastern Chinese population: a three-center case–control study

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Neuroblastoma is one of the most common malignancy in childhood, which originates from the developing sympathetic nervous system. Single nucleotide polymorphisms (SNPs) in primary miRNA (pri-miRNA) have shown to associate with cancer susceptibility, including neuroblastoma. Three precursor miRNA (pre-miRNA) SNPs (pre-miR-146a rs2910164, pre-miR-149 rs2292832 and pre-miR-196a2 rs11614913) were found to contribute to pathogenesis of various diseases. Here, to evaluate the association among these three pre-miRNA SNPs and neuroblastoma susceptibility in Eastern Chinese children, we carried out a three-center case–control study involving 312 neuroblastoma cases and 762 healthy controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the association of these three polymorphisms with neuroblastoma risk. However, no significant association was observed among these three SNPs and neuroblastoma susceptibility, in either overall or subgroups analysis by tumor sites, gender and age. Further larger studies consisting of diverse ethnic populations are required to clarify the associations among these three pre-miRNAs polymorphisms and neuroblastoma risk.

Introduction

Neuroblastoma, a common childhood extracranial tumor, originates from the developing sympathetic nervous system [1]. It mainly occurs in infancy, with the median age of 17 months at diagnosis [2]. In addition, neuroblastoma accounts for approximately 10% of all malignancies and 15% of malignancy deaths in children [3]. This type of childhood malignancy shows great heterogeneity in clinical presentation and prognosis. Neuroblastoma could be classified into low, intermediate, and high risk groups based on clinical and biological characteristics, including tumor stage, histopathology, age, DNA index, and MYCN amplification [4–6].

Moreover, several genome-wide association studies (GWASs) have identified extra neuroblastoma susceptibility genes, including CASC15, BARD1, LMO1, DUSP12, HACE1, LIN28B, CPZ and RSRC1 [7–12]. Beside GWASs, candidate gene approaches also identified several candidate loci, such as, the NEFL rs1059111 polymorphism correlates with increased levels of NEFL, and confers protection against neuroblastoma development [13]. The CDKN1B rs34330 polymorphism acts as a genetic risk factor for
neuroblastoma by affecting the transcription rate [14]. The BARD1 rs17489363 and rs1048108 polymorphisms could influence neuroblastoma susceptibility [15].

MicroRNAs (miRNAs) are short non-coding RNAs (approximately 17–22 nucleotides in length). These small RNAs can bind to the 3′-untranslated region (3′-UTR) of gene transcripts to induce target mRNA degradation or inhibit translation [16]. miRNAs play important roles in carcinogenesis, including neuroblastoma [17–20]. DNA sequences encoding miRNAs include single miRNA gene loci, miRNA gene clusters, or fragments within introns of protein-coding genes. The primary miRNA (pri-miRNA) are processed to generate small mature miRNAs by the RNase III enzymes, Dicer and Drosha [21]. The single nucleotide polymorphisms (SNPs) in precursor miRNA (pre-miRNA) encoding sequences may influence miRNA expression and maturation, thereby modifying susceptibility to neuroblastoma and other cancers [22–26].

Pre-miR-146a, pre-miR-149 and pre-miR-196a2 have been reported as key factors for carcinogenesis due to their targeting on several vital genes [27,28]. The pre-miR-146a rs2910164, pre-miR-149 rs2292832 and pre-miR-196a2 rs11614913 polymorphisms could influence their functions through altering miRNA maturation, expression, and/or efficiency of targeting [29]. Previous studies showed that miR-146a was related to regulation of TNF-α [30], miR-149 could regulate the expression of MTHFR [31], and miR-196a2 could target ANXA1, DFFA and PDCD4 [32]. These three well-known pre-miRNAs polymorphisms have been reported to be associated with the pathogenesis of various diseases, such as breast cancer, colorectal cancer, hepatocellular carcinoma, and gastric cancer [33–38]. However, in our previous studies in Chinese children of Southern and North China, no significant association among these three pre-miRNAs’ polymorphisms and neuroblastoma susceptibility was observed [25]. Herein, we performed a three-center hospital-based case–control study to test the association among these three pre-miRNAs’ SNPs and neuroblastoma risk with 312 cases and 762 controls residing in East China.

Materials and methods

Study subjects

Participants were recruited from three hospitals: Children’s Hospital of Nanjing Medical University (158 cases and 426 controls, Jiangsu province, China) [39], Anhui Provincial Children’s Hospital (119 cases and 264 controls, Anhui province, China) [40], and The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University (36 cases and 72 controls, Wenzhou area) [41,42]. A total of 313 cases and 762 controls were enrolled for the case–control study (Supplementary Table S1). The study was approved by the institutional review boards of Children’s Hospital of Nanjing Medical University, Anhui Provincial Children’s Hospital and The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University. The selection standard and details of the included participants are accessible in our previous studies [43–46]. All participants or their guardians provided written informed consent before the study.

SNPs selection and genotyping

Three well-known polymorphisms (miR-146a rs2910164, miR-149 rs2292832 and miR-196a2 rs11614913) were selected. An online tool, SNPinfo (http://snpinfo.niehs.nih.gov/) was used to predict the functions of selected SNPs (Supplementary Table S2). DNA samples were extracted as previously described [45]. Briefly, DNA samples were diluted to a stock concentration of 10 ng/μl and added to 96-well plates. Genotyping for these three polymorphisms was carried out in a 384-well plate using TaqMan Real-Time PCR method [47,48]. Moreover, 10% of the samples were randomly chosen for duplicate analyses and the results of quality control samples were 100% concordant.

Statistical analysis

Deviation from Hardy–Weinberg equilibrium (HWE) in controls was calculated for the selected SNPs by goodness-of-fit χ² test. Differences in the genotypes and demographics between cases and controls were assessed by χ² test. To evaluate the association among the three selected SNPs and neuroblastoma susceptibility, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a multivariate logistic regression. Moreover, stratified analyses were evaluated by tumor sites, gender and age. A P < 0.05 was considered as statistically significant. All statistical tests were calculated using SAS software (v9.4; SAS Institute, Cary, NC, U.S.A.).
Table 1: Association between selected miRNAs polymorphisms and neuroblastoma risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n=313)</th>
<th>Controls (n=762)</th>
<th>P1</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR146a rs2910164 C&gt;G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>114 (36.42)</td>
<td>272 (35.70)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>159 (50.80)</td>
<td>365 (47.90)</td>
<td>1.04 (0.78–1.39)</td>
<td>0.792</td>
<td>1.05 (0.79–1.40)</td>
<td>0.758</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>40 (12.78)</td>
<td>125 (16.40)</td>
<td>0.76 (0.50–1.16)</td>
<td>0.206</td>
<td>0.76 (0.50–1.16)</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.314</td>
<td>0.91 (0.75–1.11)</td>
<td>0.345</td>
<td>0.91 (0.75–1.11)</td>
<td>0.345</td>
</tr>
<tr>
<td>Dominant</td>
<td>199 (63.58)</td>
<td>490 (64.30)</td>
<td>0.822</td>
<td>0.97 (0.74–1.27)</td>
<td>0.821</td>
<td>0.97 (0.74–1.28)</td>
<td>0.844</td>
</tr>
<tr>
<td>Recessive</td>
<td>270 (87.22)</td>
<td>637 (83.60)</td>
<td>0.134</td>
<td>0.75 (0.51–1.10)</td>
<td>0.135</td>
<td>0.74 (0.51–1.09)</td>
<td>0.125</td>
</tr>
<tr>
<td>miR-149 rs2292832 T&gt;C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>226 (72.20)</td>
<td>542 (71.13)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>68 (21.73)</td>
<td>161 (21.13)</td>
<td>1.01 (0.73–1.40)</td>
<td>0.938</td>
<td>1.01 (0.73–1.40)</td>
<td>0.944</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19 (6.07)</td>
<td>59 (7.74)</td>
<td>0.77 (0.45–1.33)</td>
<td>0.348</td>
<td>0.78 (0.45–1.34)</td>
<td>0.362</td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.629</td>
<td>0.93 (0.75–1.16)</td>
<td>0.504</td>
<td>0.93 (0.75–1.16)</td>
<td>0.515</td>
</tr>
<tr>
<td>Dominant</td>
<td>87 (27.80)</td>
<td>220 (28.87)</td>
<td>0.723</td>
<td>0.95 (0.71–1.27)</td>
<td>0.724</td>
<td>0.95 (0.71–1.27)</td>
<td>0.728</td>
</tr>
<tr>
<td>Recessive</td>
<td>294 (93.93)</td>
<td>703 (92.26)</td>
<td>0.337</td>
<td>0.77 (0.45–1.31)</td>
<td>0.338</td>
<td>0.78 (0.45–1.33)</td>
<td>0.353</td>
</tr>
<tr>
<td>miR-196a2 rs11614913 T&gt;C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>89 (28.43)</td>
<td>230 (30.18)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>163 (52.08)</td>
<td>377 (49.48)</td>
<td>1.12 (0.82–1.52)</td>
<td>0.477</td>
<td>1.12 (0.82–1.52)</td>
<td>0.478</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>61 (19.49)</td>
<td>155 (20.34)</td>
<td>1.02 (0.69–1.49)</td>
<td>0.931</td>
<td>1.02 (0.69–1.50)</td>
<td>0.922</td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.738</td>
<td>1.02 (0.84–1.23)</td>
<td>0.848</td>
<td>1.02 (0.84–1.23)</td>
<td>0.840</td>
</tr>
<tr>
<td>Dominant</td>
<td>224 (71.57)</td>
<td>532 (69.82)</td>
<td>0.568</td>
<td>1.09 (0.81–1.46)</td>
<td>0.569</td>
<td>1.09 (0.81–1.46)</td>
<td>0.566</td>
</tr>
<tr>
<td>Recessive</td>
<td>252 (80.51)</td>
<td>607 (79.66)</td>
<td>0.751</td>
<td>0.95 (0.68–1.32)</td>
<td>0.751</td>
<td>0.95 (0.68–1.32)</td>
<td>0.762</td>
</tr>
</tbody>
</table>

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

Results

Participants’ characteristics

The clinical and demographic characteristics of 313 neuroblastoma cases and 762 controls are shown in Supplementary Table S1. There were no significant differences between neuroblastoma cases and controls in age (P=0.823) and gender (P=0.610).

Association between selected miRNA polymorphisms and neuroblastoma risk

As shown in Table 1 and Supplementary Table S3, the genotype counts and association results of these three pre-miRNAs’ polymorphisms (pre-miR-146a rs2910164, pre-miR-149 rs2292832 and pre-miR-196a2 rs11614913) were assessed. All of the three selected SNPs were in accordance with the HWE in controls. However, no significant association was observed among the three selected SNPs and neuroblastoma risk in the combined study population.

Stratified analysis

Next, participants were stratified in terms of tumor sites, gender, and age. Consistently, as shown in Table 2, there was no significant association for these three pre-miRNAs polymorphisms in subgroups (defined by tumor sites, gender, and age).

Discussion

In this three-center case–control study of neuroblastoma, we investigated the association of three well-known pre-miRNAs’ polymorphisms with neuroblastoma risk in Eastern Chinese children. To our knowledge, this is the first replication study for the first time in Eastern Chinese children, regarding such association.

miRNAs could negatively regulate gene expression by binding 3′-UTR of target mRNAs and subsequently affect cell differentiation, proliferation [49]. miR-146a rs2910164 G>C, miR-149 rs2292832 C>T and miR-196a2 rs11614913 T>C polymorphisms have been reported to be associated with the risk of breast, lung, thyroid, and gastric cancer [50,51]. Further studies found that the miR-196a2 rs11614913 polymorphism was significantly associated...
Table 2 Stratification analysis for the association between selected polymorphism and neuroblastoma susceptibility

<table>
<thead>
<tr>
<th>Variables</th>
<th>rs2910164 (case/control)</th>
<th>AOR (95% CI)</th>
<th>P</th>
<th>rs2292832 (case/control)</th>
<th>AOR (95% CI)</th>
<th>P</th>
<th>rs11614913 (case/control)</th>
<th>AOR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC/CG</td>
<td></td>
<td></td>
<td>TT</td>
<td></td>
<td></td>
<td>TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 18</td>
<td>124/283</td>
<td>0.73 (0.41–1.29)</td>
<td>0.271</td>
<td>101/243</td>
<td>1.03 (0.67–1.59)</td>
<td>0.890</td>
<td>43/99</td>
<td>0.96 (0.62–1.47)</td>
<td>0.840</td>
</tr>
<tr>
<td>&gt; 18</td>
<td>149/354</td>
<td>0.77 (0.46–1.29)</td>
<td>0.318</td>
<td>125/299</td>
<td>0.89 (0.60–1.20)</td>
<td>0.575</td>
<td>46/131</td>
<td>1.22 (0.82–1.82)</td>
<td>0.318</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>127/286</td>
<td>0.75 (0.42–1.34)</td>
<td>0.328</td>
<td>108/247</td>
<td>0.93 (0.59–1.45)</td>
<td>0.731</td>
<td>47/101</td>
<td>0.90 (0.59–1.37)</td>
<td>0.610</td>
</tr>
<tr>
<td></td>
<td>146/351</td>
<td>0.75 (0.45–1.25)</td>
<td>0.271</td>
<td>118/295</td>
<td>0.99 (0.67–1.46)</td>
<td>0.947</td>
<td>42/129</td>
<td>1.33 (0.88–1.99)</td>
<td>0.174</td>
</tr>
<tr>
<td>Sites of origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>62/637</td>
<td>0.49 (0.21–1.16)</td>
<td>0.103</td>
<td>50/542</td>
<td>0.88 (0.50–1.55)</td>
<td>0.688</td>
<td>18/230</td>
<td>1.19 (0.68–2.09)</td>
<td>0.538</td>
</tr>
<tr>
<td>Retropertioneal</td>
<td>107/637</td>
<td>0.90 (0.53–1.52)</td>
<td>0.691</td>
<td>91/542</td>
<td>0.96 (0.63–1.46)</td>
<td>0.833</td>
<td>37/230</td>
<td>1.04 (0.69–1.57)</td>
<td>0.855</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>87/637</td>
<td>0.70 (0.37–1.32)</td>
<td>0.271</td>
<td>71/542</td>
<td>0.98 (0.61–1.55)</td>
<td>0.920</td>
<td>28/230</td>
<td>1.09 (0.69–1.74)</td>
<td>0.706</td>
</tr>
<tr>
<td>Others</td>
<td>17/637</td>
<td>0.88 (0.25–3.05)</td>
<td>0.836</td>
<td>14/542</td>
<td>1.07 (0.41–2.83)</td>
<td>0.893</td>
<td>6/230</td>
<td>0.98 (0.37–2.59)</td>
<td>0.966</td>
</tr>
</tbody>
</table>

Abbreviation: AOR, adjusted OR; CI, confidence interval.

1Adjusted for age and gender, omitting the corresponding stratification factor.
with the developing cancer risk [52,53]. According to recent reports, the miR-146a, miR-149, and miR-196a2 polymorphisms may be genetic predisposing factors in different diseases [33,34,54]. The miR-146a, miR-149 and miR-196a2 polymorphisms were significantly associated with the expression of TNF-α, Annexin A1, C-reactive protein, and MTHFR [50]. However, in our previous study in South and North China, no significant association among these three pre-miRNAs polymorphisms (miR146a rs2910164, miR-149 rs2298232, and miR-196a2 rs11614913) and neuroblastoma susceptibility was observed [25].

Herein, we also observed no significant association of these three pre-miRNAs SNPs with neuroblastoma risk in the Eastern Chinese populations. Several limitations existed in the present study. First, the present study population only included Eastern Chinese individuals, and the results need to be validated in other ethnic groups. Second, the statistical power may be limited due to small sample size. Third, because of the retrospective study, selection and information bias might be unavoidable. Finally, most SNPs only have weak effects on tumor risk [55,56]. Only three polymorphisms were investigated in the present study. Some potentially functional SNPs remain to be assessed in the future.

In conclusion, our study showed that three pre-miRNAs polymorphisms (pre-miR-146a rs2910164, pre-miR-149 rs2298232 and pre-miR-196a2 rs11614913) are not associated with neuroblastoma risk in Eastern Chinese children. Therefore, additional studies with larger sample size are required to fully understand the role of the three pre-miRNAs’ polymorphisms in neuroblastoma susceptibility.

Funding
This work was supported by the Pearl River S&T Nova Program of Guangzhou [grant number 201710010086].

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

Author Contribution
Jing He and Haiyan Wu designed the experiments, supervised the project, and was involved in all aspects of the submission. Chunlei Zhou, Jinhong Zhu, Yingzi Tang, Lili He, Jinghang Li, Yizhen Wang and Haixia Zhou performed the experiments and participated in the study design, data analysis, and manuscript preparation. All authors read and approved the final manuscript.

Abbreviations
CI, confidence interval; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; miRNA, microRNA; OR, odds ratio; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; SNP, single nucleotide polymorphism; UTR, untranslated region.

References


22 Sun, J.H., Kim, S.H., Yang, W.I., Kim, W.J., Moon, J.Y. et al. (2016) miRNA polymorphisms (miR146a, miR149, miR196a2 and miR499) are associated with the risk of coronary artery disease. Mol. Rep. 14, 2328–2342, https://doi.org/10.3892/mmr.2016.5495


