Associations between WTAP gene polymorphisms and neuroblastoma susceptibility in Chinese children

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Background: Previous studies have revealed that WTAP is related to multiple types of cancer. Recently, WTAP has been reported as an independent prognostic factor in patients with neuroblastoma.

Methods: To explore the association between three WTAP polymorphisms (rs9457712 G>A, rs1853259 A>G and rs7766006 G>T) and neuroblastoma susceptibility in Chinese populations, we performed this case-control study including 898 neuroblastoma cases and 1,734 controls. We genotyped these potentially functional single nucleotide polymorphisms (SNPs) by TaqMan assays. The odds ratios (ORs) and 95% confidence intervals (CIs) by logistic regression models were used to assess the relationship between WTAP SNPs and the risk of neuroblastoma.

Results: No significant associations were observed in the overall analysis between any of the three WTAP polymorphisms and the risk of neuroblastoma. However, in the age ≤18 months subgroup, we found that the rs1853259 AG/GG genotype exerted protective effects against neuroblastoma (adjusted OR =0.77, 95% CI: 0.59–0.998, P=0.048), whereas the presence of 1–2 combined risk genotypes significantly increased the risk of neuroblastoma (adjusted OR =1.32, 95% CI: 1.02–1.71, P=0.036).

Conclusions: WTAP gene polymorphisms only have a weak impact on the risk of neuroblastoma in the Chinese children. Further case-control studies, preferable on larger sample sizes, are needed to validate our results.

Keywords: WTAP; m6A; polymorphism; neuroblastoma; susceptibility


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Introduction

Neuroblastoma is an extracranial solid tumor derived from neural crest tissues, accounting for about 15% of pediatric tumor-related mortality (1). As one of the most common solid malignancies in children, neuroblastoma exhibits diverse clinical behaviors. The survival rate for patients with high-risk tumors is lower than 50% even after receiving multimodality treatment, while some patients undergo spontaneous regression after mild or no treatment (1,2). The pathogenesis of neuroblastoma is multifactorial and remains far from clear. Emerging evidence shows that the transformation from normal cells to tumor cells is attributed to a gradual accumulation of genetic alterations (2-4). It is imperative to reveal the genetic mechanisms of neuroblastoma formation, which has the potential to provide novel therapeutic approaches for refractory neuroblastoma. Advances in genome-wide association studies (GWASs) allow the detection of genetic variations in tumor samples and result in significant progress in the understanding of the heritability of neuroblastoma (4,5). At present, many genetic and epigenetic variations that not only contribute to tumorigenesis but also promote the malignant potential of neuroblastoma have been demonstrated by GWASs (6-8). Single nucleotide polymorphisms (SNPs) within HSD17B12, DDX4, and DUSP12 are enriched in patients with low-risk neuroblastoma (4,5,9). SNPs in LMO1, CASC15, and LIN28B are significantly correlated with high-risk neuroblastoma and are involved in promoting proliferation and invasion (8,10,11).

Wilms’ tumor 1-associating protein (WTAP), located at chromosome region 6q25-27, is involved in regulating embryonic development, cell proliferation and apoptosis (12,13). WTAP has also been identified as an oncogenic protein in diffuse large B-cell lymphoma and acute myeloid leukemia (14,15). Moreover, accumulating evidence indicates that WTAP plays an important role in the initiation and development of various human malignancies, including glioma, ovarian cancer, renal cell carcinoma and pancreatic ductal adenocarcinoma (16-18). The role of WTAP SNPs on the cancer susceptibility also has been investigated. Our research group have identified a significant relationship between rs7766006 and hepatoblastoma risk in the Chinese population (19). However, no study has been reported to evaluate the associations between WTAP SNPs and neuroblastoma susceptibility.

To assess the associations between the SNPs in WTAP and neuroblastoma risk, we carried out this case-control study of 898 neuroblastoma patients and 1,734 control subjects using a Chinese population of children. We present the following article/case in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/tp-20-168).

Methods

Study subjects

Here, we totally enrolled 898 neuroblastoma patients and 1,734 controls from eight hospitals from eight cities (Guangzhou, Zhengzhou, Wenzhou, Xi’an, Taiyuan, Kunming, Changsha, Shenyang) in China (Table S1). All the enrolled subjects were genetically unrelated and of Chinese descents. Age, sex, and ethnicity were well matched in the patients and controls. Neuroblastoma patients were diagnosed by biopsy and staged based on the International Neuroblastoma Staging System (INSS) (20). Each participant’s parents or guardians provided written informed consent. This study was approved by the Institutional Review Board of Guangzhou Women and Children’s Medical Center (No: 201929300). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Polymorphism selection and genotyping

Potential functional polymorphisms in the WTAP gene were searched in the dbSNP database (http://www.ncbi.nlm.nih.gov/) and SNPinfo (http://snpinfo.niehs.nih.gov/) according to the selection criteria described in our reported publication (21,22). Three SNPs (rs9457712 G>A, rs1853259 A>G and rs7766006 G>T) in the WTAP gene were eventually selected (23). These SNPs were detected by standard TaqMan real-time PCR (24-26). To assure the accuracy of genotyping results, 10% of the samples were selected randomly to run a second genotype. All repeated samples were 100% concordant.

Statistical analysis

Differences in genotype distribution and demographic characteristics between patients and controls were compared by two-sided \( \chi^2 \) tests. Hardy-Weinberg equilibrium (HWE) for the selected SNPs in controls was assessed by a goodness-of-fit \( \chi^2 \) test. Associations between neuroblastoma susceptibility and WTAP SNPs were evaluated using odds
ratios (ORs) and 95% confidence intervals (CIs). Stratified analysis was conducted regarding age, sex, tumor sites, and clinical stages. P<0.05 was considered statistically significant. All statistical analyses were carried out using SAS software (Version 9.4; SAS Institute, Cary, NC, USA).

Results

WTAP gene polymorphisms and neuroblastoma susceptibility

In the current study, 896 cases and 1,732 controls were successfully genotyped. The genotype distribution of the three WTAP polymorphisms and their associations with neuroblastoma susceptibility are revealed in Table 1. All these SNPs were in accordance with HWE among the control subjects (P=0.213 for the rs9457712 G>A polymorphism, P=0.185 for the rs1853259 A>G polymorphism, and P=0.799 for the rs7766006 G>T polymorphism). No significant associations were detected between the selected WTAP SNPs and neuroblastoma susceptibility.

Stratification analysis

We further divided participants into subgroups based on sex, age, sites of tumor origin, and clinical stages. The effects of the selected SNPs on neuroblastoma risk were determined in this stratified analysis (Table 2). Our results indicated that children ≤18 months old with rs1853259 AG/GG genotypes were less likely to develop neuroblastoma (OR =0.77, 95% CI: 0.59–0.998, P=0.048). However, children ≤18 months old harboring 1–2 combined risk genotypes had increased neuroblastoma susceptibility (OR =1.32, 95% CI: 1.02–1.71, P=0.036).

Discussion

We performed this eight-center study to investigate the association between WTAP gene polymorphisms and neuroblastoma susceptibility. Our data manifested that rs1853259 AG/GG genotypes were correlated with a decreased neuroblastoma risk in children ≤18 months old. However, children ≤18 months old harboring 1–2 combined risk genotypes are more likely to develop neuroblastoma. To our knowledge, the current study represents the first to explore the association between WTAP SNPs and neuroblastoma susceptibility.

WTAP was initially identified as a nuclear protein and is involved in N6-methyladenosine RNA modification, which affects the initiation and progression of several human malignancies by modulating the mRNA expression of oncogene genes (12,27-29). In addition, WTAP can also execute oncogenic effects by inhibiting apoptosis, accelerating proliferation and promoting invasion of malignant cells (12,14,30). Previous studies have demonstrated that overexpression of WTAP is associated with poor survival in renal cell carcinoma, gastric cancer and pancreatic ductal adenocarcinoma (31-33).

Given the vital role of WTAP in the initiation and progression of malignancies, investigation into the association between WTAP SNPs and neuroblastoma susceptibility is warranted. Therefore, we conducted this study to explore the association between WTAP SNPs and neuroblastoma risk in Chinese children. In the current study, no significant associations were discovered in the overall analysis between the selected WTAP SNPs and neuroblastoma susceptibility. However, in the age ≤18 months subgroup, we found that rs1853259 AG/GG genotypes exerted protective effects against neuroblastoma, whereas the presence of 1–2 combined risk genotypes significantly increased the risk of neuroblastoma.

There are several limitations present in our current study. First, neuroblastoma is a remarkably heterogeneous disease with a complex etiology. However, several confounding factors, including dietary intake and living environment, were not assessed in our current study. The results should be explained with caution in the absence of other confounding factors. Further comprehensive study incorporating the combined analysis of genetic factors and confounding factors are warranted. Second, here we only analyzed three WTAP SNPs. Further investigation will be required to uncover more polymorphisms that predispose patients to neuroblastoma, which may provide novel insights into the genetic etiology of neuroblastoma. Third, ethnic background may affect genetic predisposition. Our results based on Chinese populations may not be directly extrapolated to other ethnicities. Fourth, the negative results might be attributed to the relatively small sample size in our current study, which might not be large enough to detect an association.

In summary, our study found that none of the WTAP polymorphisms (rs9457712 G>A, rs1853259 A>G and rs7766006 G>T) were related to neuroblastoma susceptibility in the overall analysis. The effect of WTAP SNPs on neuroblastoma predisposition must be elucidated.
Table 1  Association between WTAP gene polymorphisms and neuroblastoma risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (N=896)</th>
<th>Controls (N=1,732)</th>
<th>Crude OR (95% CI)</th>
<th>P*</th>
<th>Adjusted OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9457712 G&gt;A (HWE =0.213)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>601 (67.08)</td>
<td>1,167 (67.38)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>GA</td>
<td>259 (28.91)</td>
<td>500 (28.87)</td>
<td>1.01 (0.84–1.20)</td>
<td>0.949</td>
<td>1.00 (0.84–1.20)</td>
<td>0.963</td>
</tr>
<tr>
<td>AA</td>
<td>36 (4.02)</td>
<td>65 (3.75)</td>
<td>1.08 (0.71–1.64)</td>
<td>0.731</td>
<td>1.10 (0.72–1.67)</td>
<td>0.670</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.804</td>
<td>1.02 (0.88–1.18)</td>
<td>0.804</td>
<td>1.02 (0.88–1.18)</td>
</tr>
<tr>
<td>Dominant</td>
<td>295 (32.92)</td>
<td>565 (32.62)</td>
<td>0.875</td>
<td>1.01 (0.85–1.20)</td>
<td>0.875</td>
<td>1.02 (0.85–1.21)</td>
</tr>
<tr>
<td>Recessive</td>
<td>860 (95.98)</td>
<td>1,667 (96.25)</td>
<td>0.738</td>
<td>1.07 (0.71–1.63)</td>
<td>0.735</td>
<td>1.09 (0.72–1.66)</td>
</tr>
</tbody>
</table>

rs1853259 A>G (HWE =0.185)  

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (N=896)</th>
<th>Controls (N=1,732)</th>
<th>Crude OR (95% CI)</th>
<th>P*</th>
<th>Adjusted OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>333 (37.17)</td>
<td>624 (36.03)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>431 (48.10)</td>
<td>853 (49.25)</td>
<td>0.95 (0.79–1.13)</td>
<td>0.543</td>
<td>0.94 (0.79–1.12)</td>
<td>0.476</td>
</tr>
<tr>
<td>GG</td>
<td>132 (14.73)</td>
<td>255 (14.72)</td>
<td>0.97 (0.76–1.24)</td>
<td>0.809</td>
<td>0.96 (0.75–1.23)</td>
<td>0.736</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.688</td>
<td>0.98 (0.87–1.10)</td>
<td>0.688</td>
<td>0.97 (0.86–1.09)</td>
</tr>
<tr>
<td>Dominant</td>
<td>563 (62.83)</td>
<td>1,108 (63.97)</td>
<td>0.566</td>
<td>0.95 (0.81–1.13)</td>
<td>0.565</td>
<td>0.94 (0.80–1.11)</td>
</tr>
<tr>
<td>Recessive</td>
<td>764 (85.27)</td>
<td>1,477 (85.28)</td>
<td>0.995</td>
<td>1.00 (0.80–1.26)</td>
<td>0.995</td>
<td>0.99 (0.79–1.25)</td>
</tr>
</tbody>
</table>

rs7766006 G>T (HWE =0.799)  

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (N=896)</th>
<th>Controls (N=1,732)</th>
<th>Crude OR (95% CI)</th>
<th>P*</th>
<th>Adjusted OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>304 (33.93)</td>
<td>584 (33.72)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>GT</td>
<td>430 (47.99)</td>
<td>839 (48.44)</td>
<td>0.99 (0.82–1.18)</td>
<td>0.866</td>
<td>0.99 (0.82–1.18)</td>
<td>0.870</td>
</tr>
<tr>
<td>TT</td>
<td>162 (18.08)</td>
<td>309 (17.84)</td>
<td>1.01 (0.80–1.27)</td>
<td>0.953</td>
<td>1.02 (0.80–1.29)</td>
<td>0.898</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td>0.992</td>
<td>1.00 (0.89–1.12)</td>
<td>0.992</td>
<td>1.00 (0.90–1.13)</td>
</tr>
<tr>
<td>Dominant</td>
<td>592 (66.07)</td>
<td>1,148 (66.28)</td>
<td>0.914</td>
<td>0.99 (0.84–1.18)</td>
<td>0.914</td>
<td>0.99 (0.84–1.18)</td>
</tr>
<tr>
<td>Recessive</td>
<td>734 (81.92)</td>
<td>1,423 (82.16)</td>
<td>0.879</td>
<td>1.02 (0.82–1.25)</td>
<td>0.879</td>
<td>1.03 (0.83–1.26)</td>
</tr>
</tbody>
</table>

Combined effect of risk genotypes*  

<table>
<thead>
<tr>
<th>Cases (N=896)</th>
<th>Controls (N=1,732)</th>
<th>Crude OR (95% CI)</th>
<th>P*</th>
<th>Adjusted OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>429 (47.88)</td>
<td>850 (49.08)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>144 (16.07)</td>
<td>266 (15.36)</td>
<td>1.07 (0.85–1.36)</td>
<td>0.555</td>
<td>1.07 (0.85–1.35)</td>
</tr>
<tr>
<td>2</td>
<td>323 (36.05)</td>
<td>616 (35.57)</td>
<td>1.04 (0.87–1.24)</td>
<td>0.674</td>
<td>1.05 (0.88–1.25)</td>
</tr>
<tr>
<td>1–2</td>
<td>467 (52.12)</td>
<td>882 (50.92)</td>
<td>0.561</td>
<td>1.05 (0.89–1.23)</td>
<td>0.561</td>
</tr>
</tbody>
</table>

* χ^2 test for genotype distributions between neuroblastoma patients and cancer-free controls; ^b, adjusted for age and gender; ^c, risk genotypes were rs9457712 GA/AA, rs1853259 AA and rs7766006 TT. OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
Table 2 Stratification analysis for association between WTAP gene genotypes and neuroblastoma susceptibility

<table>
<thead>
<tr>
<th>Variables</th>
<th>rs9457712 (case/control)</th>
<th>rs1853259 (case/control)</th>
<th>rs7766006 (case/control)</th>
<th>Risk genotypes (case/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA/AA</td>
<td>AOR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age, month</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤18</td>
<td>218/482</td>
<td>126/231</td>
<td>1.21 (0.92–1.58)</td>
<td>0.175</td>
</tr>
<tr>
<td>&gt;18</td>
<td>383/685</td>
<td>169/334</td>
<td>0.91 (0.73–1.14)</td>
<td>0.418</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>275/510</td>
<td>131/234</td>
<td>1.04 (0.80–1.35)</td>
<td>0.786</td>
</tr>
<tr>
<td>Male</td>
<td>326/657</td>
<td>164/331</td>
<td>1.00 (0.79–1.26)</td>
<td>0.985</td>
</tr>
<tr>
<td>Sites of origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>165/1,167</td>
<td>83/565</td>
<td>1.03 (0.78–1.37)</td>
<td>0.826</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>219/1,167</td>
<td>99/565</td>
<td>0.93 (0.72–1.20)</td>
<td>0.559</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>138/1,167</td>
<td>75/565</td>
<td>1.14 (0.84–1.53)</td>
<td>0.406</td>
</tr>
<tr>
<td>Others</td>
<td>72/1,167</td>
<td>33/565</td>
<td>0.95 (0.62–1.46)</td>
<td>0.825</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I + II +4 s</td>
<td>317/1,167</td>
<td>152/565</td>
<td>1.00 (0.80–1.24)</td>
<td>0.972</td>
</tr>
<tr>
<td>III + IV</td>
<td>265/1,167</td>
<td>129/565</td>
<td>1.01 (0.80–1.28)</td>
<td>0.944</td>
</tr>
</tbody>
</table>

* adjusted for age and gender, omitting the corresponding stratify factor. AOR, adjusted odds ratio; CI, confidence interval.
by well-designed studies.

**Acknowledgments**

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**Footnote**

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at [http://dx.doi.org/10.21037/tp-20-168](http://dx.doi.org/10.21037/tp-20-168)

*Data Sharing Statement:* Available at [http://dx.doi.org/10.21037/tp-20-168](http://dx.doi.org/10.21037/tp-20-168)

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at [http://dx.doi.org/10.21037/tp-20-168](http://dx.doi.org/10.21037/tp-20-168)). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was acquired from the parents or guardians of each participant. This study was approved by the Institutional Review Board of Guangzhou Women and Children’s Medical Center (No: 201929300).

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Combined subjects (8 Centers)</th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (N=898)</td>
<td>Controls (N=1734)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age range, month</td>
<td>0.00-176.00</td>
<td>0.004-156.00</td>
<td></td>
<td>0.155</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>33.11±28.07</td>
<td>30.41±24.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤18</td>
<td>344</td>
<td>38.31</td>
<td>714</td>
<td>41.18</td>
</tr>
<tr>
<td>&gt;18</td>
<td>554</td>
<td>61.69</td>
<td>1020</td>
<td>58.82</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.236</td>
</tr>
<tr>
<td>Female</td>
<td>407</td>
<td>45.32</td>
<td>744</td>
<td>42.91</td>
</tr>
<tr>
<td>Male</td>
<td>491</td>
<td>54.68</td>
<td>990</td>
<td>57.09</td>
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SD, standard deviation; NA, not available. * Two-sided \( \chi^2 \) test for distributions between neuroblastoma cases and cancer-free controls.