



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

Submitted Jul 01, 2020. Accepted for publication Oct 30, 2020.

doi: 10.21037/tp-20-168

View this article at: <http://dx.doi.org/10.21037/tp-20-168>

Introduction

Neuroblastoma is a 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://snpinfonihs.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 χ^2 tests. Hardy-Weinberg equilibrium (HWE) for the selected SNPs in controls was assessed by a goodness-of-fit χ^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 are 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

Genotype	Cases (N=896)	Controls (N=1,732)	P ^a	Crude OR (95% CI)	P	Adjusted OR (95% CI) ^b	P ^b
rs9457712 G>A (HWE =0.213)							
GG	601 (67.08)	1,167 (67.38)		1.00		1.00	
GA	259 (28.91)	500 (28.87)		1.01 (0.84–1.20)	0.949	1.00 (0.84–1.20)	0.963
AA	36 (4.02)	65 (3.75)		1.08 (0.71–1.64)	0.731	1.10 (0.72–1.67)	0.670
Additive			0.804	1.02 (0.88–1.18)	0.804	1.02 (0.88–1.18)	0.775
Dominant	295 (32.92)	565 (32.62)	0.875	1.01 (0.85–1.20)	0.875	1.02 (0.85–1.21)	0.869
Recessive	860 (95.98)	1,667 (96.25)	0.738	1.07 (0.71–1.63)	0.735	1.09 (0.72–1.66)	0.671
rs1853259 A>G (HWE =0.185)							
AA	333 (37.17)	624 (36.03)		1.00		1.00	
AG	431 (48.10)	853 (49.25)		0.95 (0.79–1.13)	0.543	0.94 (0.79–1.12)	0.476
GG	132 (14.73)	255 (14.72)		0.97 (0.76–1.24)	0.809	0.96 (0.75–1.23)	0.736
Additive			0.688	0.98 (0.87–1.10)	0.688	0.97 (0.86–1.09)	0.605
Dominant	563 (62.83)	1,108 (63.97)	0.566	0.95 (0.81–1.13)	0.565	0.94 (0.80–1.11)	0.488
Recessive	764 (85.27)	1,477 (85.28)	0.995	1.00 (0.80–1.26)	0.995	0.99 (0.79–1.25)	0.958
rs7766006 G>T (HWE =0.799)							
GG	304 (33.93)	584 (33.72)		1.00		1.00	
GT	430 (47.99)	839 (48.44)		0.99 (0.82–1.18)	0.866	0.99 (0.82–1.18)	0.870
TT	162 (18.08)	309 (17.84)		1.01 (0.80–1.27)	0.953	1.02 (0.80–1.29)	0.898
Additive			0.992	1.00 (0.89–1.12)	0.992	1.00 (0.90–1.13)	0.943
Dominant	592 (66.07)	1,148 (66.28)	0.914	0.99 (0.84–1.18)	0.914	0.99 (0.84–1.18)	0.938
Recessive	734 (81.92)	1,423 (82.16)	0.879	1.02 (0.82–1.25)	0.879	1.03 (0.83–1.26)	0.821
Combined effect of risk genotypes ^c							
0	429 (47.88)	850 (49.08)		1.00		1.00	
1	144 (16.07)	266 (15.36)		1.07 (0.85–1.36)	0.555	1.07 (0.85–1.35)	0.577
2	323 (36.05)	616 (35.57)		1.04 (0.87–1.24)	0.674	1.05 (0.88–1.25)	0.601
1–2	467 (52.12)	882 (50.92)	0.561	1.05 (0.89–1.23)	0.561	1.06 (0.90–1.24)	0.518

^a, 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

Variables	rs9457712 (case/control)			rs1853259 (case/control)			rs7766006 (case/control)			Risk genotypes (case/control)						
	GG	GA/AA	AOR (95% CI) ^a	P ^a	AA	AG/GG	AOR (95% CI) ^a	P ^a	GG	GT/TT	AOR (95% CI) ^a	P ^a	0	1-2	AOR (95% CI) ^a	P ^a
Age, month																
≤18	218/482	126/231	1.21 (0.92-1.58)	0.175	146/257	198/456	0.77 (0.59-0.998)	0.048	123/234	221/479	0.88 (0.67-1.15)	0.357	145/350	199/363	1.32 (1.02-1.71)	0.036
>18	383/685	169/334	0.91 (0.73-1.14)	0.418	187/367	365/652	1.09 (0.88-1.36)	0.431	181/350	371/669	1.07 (0.86-1.33)	0.554	284/500	268/519	0.92 (0.75-1.13)	0.411
Gender																
Female	275/510	131/234	1.04 (0.80-1.35)	0.786	143/260	263/484	0.98 (0.76-1.26)	0.879	139/238	267/506	0.92 (0.71-1.19)	0.522	200/378	206/366	1.07 (0.84-1.36)	0.613
Male	326/657	164/331	1.00 (0.79-1.26)	0.985	190/364	300/624	0.92 (0.73-1.15)	0.439	165/346	325/642	1.06 (0.84-1.33)	0.614	229/472	261/516	1.05 (0.84-1.30)	0.689
Sites of origin																
Adrenal gland	165/1,167	83/565	1.03 (0.78-1.37)	0.826	89/624	159/1,108	1.01 (0.76-1.33)	0.963	92/584	156/1,148	0.88 (0.67-1.16)	0.353	122/850	126/882	0.99 (0.76-1.30)	0.954
Retropitoneal	219/1,167	99/565	0.93 (0.72-1.20)	0.559	118/624	200/1,108	0.95 (0.74-1.22)	0.678	101/584	217/1,148	1.11 (0.86-1.44)	0.419	155/850	163/882	1.01 (0.79-1.28)	0.938
Mediastinum	138/1,167	75/565	1.14 (0.84-1.53)	0.406	80/624	133/1,108	0.93 (0.69-1.24)	0.610	68/584	145/1,148	1.07 (0.79-1.45)	0.664	98/850	115/882	1.15 (0.86-1.53)	0.348
Others	72/1,167	33/565	0.95 (0.62-1.46)	0.825	42/624	63/1,108	0.84 (0.56-1.26)	0.395	38/584	67/1,148	0.89 (0.59-1.34)	0.560	49/850	56/882	1.11 (0.75-1.65)	0.605
Clinical stage																
I + II + 4 s	317/1,167	152/565	1.00 (0.80-1.24)	0.972	176/624	293/1,108	0.94 (0.76-1.16)	0.553	162/584	307/1,148	0.96 (0.77-1.19)	0.678	229/850	240/882	1.02 (0.83-1.25)	0.889
III + IV	265/1,167	129/565	1.01 (0.80-1.28)	0.944	142/624	252/1,108	0.99 (0.78-1.24)	0.903	131/584	263/1,148	1.03 (0.81-1.30)	0.809	187/850	207/882	1.08 (0.86-1.35)	0.504

^a, adjusted for age and gender, omitting the corresponding stratify factor. AOR, adjusted odds ratio; CI, confidence interval.

by well-designed studies.

Acknowledgments

Funding: This study was supported by grants from the Natural Science Foundation of Guangdong Province (No: 2019A1515010360), the National Natural Science Foundation of China (No: 81560262, 81960294), and Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease (No: 2019B030301004).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <http://dx.doi.org/10.21037/tp-20-168>

Data Sharing Statement: Available at <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>). 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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Cite this article as: Tang J, Lu H, Yang Z, Li L, Li L, Zhang J, Cheng J, Li Y, Li S, Zhou H, He J, Liu W. Associations between WTAP gene polymorphisms and neuroblastoma susceptibility in Chinese children. *Transl Pediatr* 2021;10(1):146-152. doi: 10.21037/tp-20-168

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

Variables	Combined subjects (8 Centers)				P ^a
	Cases (N=898)		Controls (N=1734)		
	No.	%	No.	%	
Age range, month	0.00-176.00		0.004-156.00		0.155
Mean ± SD	33.11±28.07		30.41±24.90		
≤18	344	38.31	714	41.18	
>18	554	61.69	1020	58.82	
Gender					0.236
Female	407	45.32	744	42.91	
Male	491	54.68	990	57.09	
INSS stages					
I	310	34.52	/	/	
II	160	17.82	/	/	
III	163	18.15	/	/	
IV	231	25.72	/	/	
4s	18	2.00	/	/	
NA	16	1.78	/	/	
Sites of origin					
Adrenal gland	248	27.62	/	/	
Retroperitoneal region	319	35.52	/	/	
Mediastinum	214	23.83	/	/	
Other region	105	11.69	/	/	
NA	12	1.34	/	/	

SD, standard deviation; NA, not available. ^a Two-sided χ^2 test for distributions between neuroblastoma cases and cancer-free controls.