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WTAP Gene Variants Confer Hepatoblastoma Susceptibility: A Seven-Center Case-Control Study

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Hepatoblastoma is a rare disease, and its etiology remains to be revealed. Wilms tumor suppressor-1-associated protein (WTAP) plays a critical role in tumorigenesis. However, whether single nucleotide polymorphisms (SNPs) of the *WTAP* gene predispose to hepatoblastoma risk awaits to be investigated. With the use of the TaqMan assay, we evaluated the genotype frequencies of three *WTAP* SNPs (rs7766006 G > T, rs9457712 G > A, and rs1853259 A > G) in Chinese children with 313 hepatoblastoma patients and 1,446 controls. Among these three SNPs, only the rs7766006 T allele exhibited a significant association with hepatoblastoma risk (GT versus GG: adjusted odds ratio [OR] = 0.70, 95% confidence interval [CI] = 0.53–0.92, p = 0.009; GT/TT versus GG: adjusted OR = 0.73, 95% CI = 0.57–0.95, p = 0.017). Combined analysis indicated that subjects with two risk genotypes showed significantly higher hepatoblastoma risk, compared to individuals without a risk genotype (adjusted OR = 1.38, 95% CI = 1.02–1.88, p = 0.037). The stratified analysis revealed that the rs1853259 GG genotype, the rs7766006 GT/TT genotype, and two risk genotypes modified hepatoblastoma risk in certain subgroups. The significant results were validated by haplotype analyses and false-positive report probability analyses. Furthermore, the expression quantitative trait locus analysis indicated that rs7766006 T was associated with decreased expression of *WTAP* mRNA. Collectively, our results suggest that *WTAP* SNPs may be genetic modifiers for the development of hepatoblastoma.

INTRODUCTION

Hepatoblastoma ranks as the most prevalent pediatric liver tumor, taking up about 80% of all children's hepatic tumors.[1,2] However, hepatoblastoma constitutes less than 1% of all pediatric malignancies.[3] Hepatoblastoma is very rare, with an incidence of about 1.2 cases per million children.[4] The 5-year survival rate has drivers to nearly 80%, due to multidisciplinary therapy treatment.[5,6] On the other hand, a significant proportion of hepatoblastoma patients may still suffer from local relapse or distant metastasis even after surgery and intensive chemotherapy.[7]

Hepatoblastomas originate from abnormal differentiation of hepatocyte precursors of epithelial lineage during embryogenesis.[8] Unlike adult hepatocellular carcinoma, the rarity of hepatoblastoma hampers the intensive investigation to its etiology. Hepatitis B virus, chronic hepatitis, and cirrhosis are significant causes of adult hepatocellular carcinoma, but not hepatoblastoma.[9] Hence, the existing epidemiological data of adult hepatocellular carcinoma could not be directly applied to hepatoblastoma.[10,11] Trisomy 18, familial adenomatous polyposis, Beckwith-Wiedemann syndrome, glycogen storage disease, and thrombocytosis are critical contributors to hepatoblastoma.[12–15] In

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addition, prematurity, low birth weight, parental tobacco use, and a fibroblast growth factor receptor 3 (*FGFR3*) mutation were reported to affect hepatoblastoma risk.[16–19] Thus far, only two case-control studies were published regarding single nucleotide polymorphisms (SNPs) and the risk of hepatoblastoma by other research groups, with fewer than 100 cases.[20,21] Moreover, our group has accomplished three molecular epidemiology studies on hepatoblastoma in Chinese children previously.[22–24] Our aim is to improve the understanding of the etiology of hepatoblastoma by characterizing crucial hepatoblastoma susceptibility genetic variants.

N6-methyladenosine (m^6A) is the most frequently distributed mRNA post-transcriptional modification.[25] The m^6A machinery is composed of a series of proteins, including methyltransferases (writers) that catalyze the transfer of a methyl group to adenine nucleotides in acceptor RNA substrates, demethylases that demethylate m^6A (erasers), and m^6A-associated RNA-binding proteins that recognize m^6A in the RNA transcript (readers).[26] The classical m^6A methyltransferases complex mainly consists of methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and Wilms tumor 1-associated protein (WTAP). METTL3 is a vital methyltransferase as an S-adenosylmethionine-binding subunit, whereas METTL14 serves as an RNA-binding scaffold for substrate recognition. WTAP interacts with METTL3 and METTL14 and then localizes them into nuclear speckles.[27] m^6A modification regulates numerous biological processes, including tumorigenesis.[28] Compelling evidence has demonstrated the involvements of METTL3, METTL14, alk B homolog 5 (ALKBH5), fat mass and obesity associated (FTO), and YTH domain-containing family protein 2 (YTHDF2) in human cancers,[29–35] including hepatoblastoma.[36,37] Nevertheless, the role of WTAP in hepatoblastoma has been poorly understood. With this in mind, we conducted a multi-center case-control study to investigate the association between functional SNPs in the *WTAP* gene and hepatoblastoma risk among children of Chinese ancestry.

RESULTS

Characteristics of the Participants

Detailed demographic and clinical characteristic information of hepatoblastoma cases (n = 313) and cancer-free controls (n = 1,446) are presented in Table S1. Both case and control groups had a similar distribution in terms of age (p = 0.251) and gender (p = 0.983). The mean age was 23.75 ± 25.93 months for cases and 25.23 ± 19.38 months for controls.

Association between the *WTAP* SNPs and Hepatoblastoma Risk

Of the included subjects, 310 cases and 1,443 controls were successfully genotyped. The associations of *WTAP* gene SNPs with hepatoblastoma risk are shown in Table 1. None of the SNPs violated Hardy-Weinberg equilibrium (HWE) in control populations (p value > 0.05 for all SNPs). First, we carried out the single-locus analysis to estimate the associations between each selected SNP and hepatoblastoma risk. We found that only the rs7766006 T allele was significantly associated with decreased hepatoblastoma risk (GT versus GG: adjusted odds ratio [OR] = 0.70, 95% confidence interval [CI] = 0.53–0.92, p = 0.009; GT/TT versus GG: adjusted OR = 0.73, 95% CI = 0.57–0.95, p = 0.017). The other two SNPs (rs9457712 G > A and rs1853259 A > G) had no association with the risk of hepatoblastoma. We further assumed rs9457712 AA, rs1853259 AG/GG, and rs7766006 GG as risk genotypes to test their combined effect on the risk of hepatoblastoma. Compared to individuals without risk genotype, those with two risk genotypes had significantly enhanced susceptibility to hepatoblastoma (adjusted OR = 1.38, 95% CI = 1.02–1.88, p = 0.037).

Stratification Analysis

Stratification analysis based on age, gender, and clinical stages was further performed (Table 2). Regarding the rs1853259 polymorphism, the GG genotype was found to be associated with hepatoblastoma risk in children under 17 months of age (adjusted OR = 1.85, 95% CI = 1.20–2.85, p = 0.005). Compared with the rs7766006 GG genotype, the GT/TT genotype decreased hepatoblastoma risk in children younger than 17 months (adjusted OR = 0.60, 95% CI = 0.43–0.85, p = 0.005), females (adjusted OR = 0.66, 95% CI = 0.45–0.99, p = 0.044), and patients with stages I + II tumors (adjusted OR = 0.67, 95% CI = 0.48–0.93, p = 0.017). After combining the risk genotypes, we observed that patients with two protective genotypes were more likely to develop hepatoblastoma in children under 17 months of age (adjusted OR = 1.63, 95% CI = 1.15–2.31, p = 0.006).

WTAP Haplotype Analyses

We further examined whether the haplotypes of the three *WTAP* gene SNPs are correlated to hepatoblastoma risk in an order of rs9457712, rs1853259, and rs7766006. As shown in Table 3, the GAT haplotype was defined as the reference group. We detected a significant elevated hepatoblastoma risk in subjects with the haplotype of AAG (adjusted OR = 1.57, 95% CI = 1.02–2.43, p = 0.041) and AGT (adjusted OR = 6.10, 95% CI = 1.35–27.69, p = 0.019).

False-Positive Report Probability (FPRP) Analysis

As shown in Table 4, we preset 0.2 as the FPRP threshold. The FPRP analysis indicated that all of the statistically significant associations remained noteworthy when a prior probability of the association was considered as 0.25, except for the association with the rs7766006 G > T (GT/TT versus GG) in the female. At the prior probability level of 0.1, significant findings for the rs7766006 G > T polymorphism remained noteworthy for all participants under the heterozygous and dominant model, as well as children under 17 months of age under the dominant model. Besides, the association with the combined-risk genotypes (2 versus 0) was also noteworthy among children under 17 months of age.

Expression Quantitative Trait Loci (eQTL) Analyses

To further assess the putative functional relevance of rs7766006 G > T, its effects on *WTAP* expression were explored using released data from Genotype-Tissue Expression (GTEx). It was found that individuals carrying the rs7766006 T genotype had significantly higher *WTAP* mRNA levels in the liver (Figure 1) and cell-cultured fibroblasts (Figure 2) than those carrying the rs7766006 G genotype. We

Table 1. The Relationship between *WTAP* Gene Polymorphisms and Hepatoblastoma Susceptibility

Genotype	Cases (n = 310)	Controls (n = 1,443)	p[a]	Crude OR (95% CI)	p	Adjusted OR (95% CI)[b]	p[b]
rs9457712 G > A (HWE = 0.452)							
GG	212 (68.39)	970 (67.22)		1.00		1.00	
GA	83 (26.77)	421 (29.18)		0.90 (0.68–1.19)	0.468	0.91 (0.69–1.20)	0.484
AA	15 (4.84)	52 (3.60)		1.32 (0.73–2.39)	0.359	1.32 (0.73–2.40)	0.357
Additive			0.984	1.00 (0.80–1.25)	0.984	1.01 (0.81–1.25)	0.968
Dominant	98 (31.61)	473 (32.78)	0.691	0.95 (0.73–1.23)	0.691	0.95 (0.73–1.24)	0.709
Recessive	295 (95.16)	1,391 (96.40)	0.303	1.36 (0.76–2.45)	0.305	1.36 (0.76–2.45)	0.305
rs1853259 A > G (HWE = 0.245)							
AA	106 (34.19)	508 (36.59)		1.00		1.00	
AG	146 (47.10)	707 (49.00)		1.03 (0.78–1.35)	0.840	1.03 (0.78–1.35)	0.861
GG	58 (18.71)	208 (14.41)		1.39 (0.97–1.99)	0.072	1.38 (0.97–1.98)	0.075
Additive			0.119	1.15 (0.96–1.38)	0.119	1.15 (0.96–1.37)	0.125
Dominant	204 (65.81)	915 (63.41)	0.426	1.11 (0.86–1.44)	0.426	1.11 (0.86–1.43)	0.442
Recessive	252 (81.29)	1,235 (85.59)	0.056	1.37 (0.99–1.88)	0.056	1.37 (0.99–1.88)	0.058
rs7766006 G > T (HWE = 0.913)							
GG	125 (40.32)	479 (33.19)		1.00		1.00	
GT	128 (41.29)	703 (48.72)		**0.70 (0.53–0.92)**	**0.010**	**0.70 (0.53–0.92)**	**0.009**
TT	57 (18.39)	261 (18.09)		0.84 (0.59–1.19)	0.316	0.84 (0.59–1.18)	0.313
Additive			0.123	0.87 (0.73–1.04)	0.123	0.87 (0.73–1.04)	0.122
Dominant	185 (59.68)	964 (66.81)	0.017	**0.74 (0.57–0.95)**	**0.017**	**0.73 (0.57–0.95)**	**0.017**
Recessive	253 (81.61)	1,182 (81.91)	0.901	1.02 (0.74–1.40)	0.901	1.02 (0.74–1.40)	0.901
Combined Effect of Risk Genotypes[c]							
0	86 (27.74)	474 (32.85)		1.00		1.00	
1	104 (33.55)	492 (34.10)		1.17 (0.85–1.59)	0.338	1.16 (0.85–1.59)	0.350
2	120 (38.71)	477 (33.06)		**1.39 (1.02–1.88)**	**0.036**	**1.38 (1.02–1.88)**	**0.037**
0–1	190 (61.29)	966 (66.94)		1.00		1.00	
2	120 (38.71)	477 (33.06)	0.057	1.28 (0.99–1.65)	0.057	1.28 (0.99–1.65)	0.057

The results were in bold if the 95% confidence intervals (CIs) excluded 1 or if p values were less than 0.05. OR, odds ratio; HWE, Hardy-Weinberg equilibrium.
[a]χ^2 test for genotype distributions between hepatoblastoma patients and cancer-free controls.
[b]Adjusted for age and gender.
[c]Risk genotypes were rs9457712 AA, rs1853259 AG/GG, and rs7766006 GG.

also evaluate the impacts of the rs7766006 G > T on the mRNA level of the neighboring genes. We found that the chaperonin T-complex 1 (*TCP1*) mRNA levels in the cultured fibroblasts with the rs7766006 T genotype were significantly higher than those in cells with the rs7766006 G genotype (Figure 3).

DISCUSSION

This work was motivated by the discovery of significant contributions of m^{6}A modification genes to cancer development. Herein, we proposed a potential contributing role of *WTAP* gene SNPs to hepatoblastoma risk. We demonstrated, for the first time, that *WTAP* gene SNPs could predispose to hepatoblastoma risk in Chinese children.

WTAP, a partner of the Wilms tumor 1 (WT1) protein, was isolated by the yeast two-hybrid system.[38] WTAP is a conserved nuclear protein. Unlike the tissue-specific expression pattern of WT1, WTAP is ubiquitously expressed in diverse tissues.[38] WTAP and WT1 are present together throughout the nucleoplasm, as well as in nuclear speckles, and partially colocalize with splicing factors.[38] WTAP has been reported to be involved in diverse cellular processes, such as m^{6}A methylation modification,[27] alternative splicing,[39] X chromosome inactivation,[40] and cell-cycle regulation.[41] Moreover, substantial evidence supports the implications of WTAP in several cancers. For instance, overexpression of WTAP promotes the invasiveness of glioblastoma through stimulating the activity of epidermal growth factor receptor (EGFR)[42] and facilitates renal cell carcinoma by binding to cyclin-dependent kinase 2 (CDK2) transcript.[43] WTAP is highly expressed in hepatocellular carcinoma and serves as a predictor of poor prognosis in hepatocellular carcinoma. Functionally, WTAP-mediated m^{6}A modification aggravates the aggressiveness

Table 2. Stratification Analysis for Association between *WTAP* Gene Genotypes and Hepatoblastoma Susceptibility

Variables	rs1853259 (Case/Control)		AOR (95% CI)[a]	p[a]	rs7766006 (Case/Control)		AOR (95% CI)[a]	p[a]	Risk Genotypes (Case/Control)		AOR (95% CI)[a]	p[a]
	AA/AG	GG			GG	GT/TT			0–1	2		
Age, Month												
<17	128/555	37/86	**1.85 (1.20–2.85)**	**0.005**	73/207	92/434	**0.60 (0.43–0.85)**	**0.005**	93/435	72/206	**1.63 (1.15–2.31)**	**0.006**
≥17	124/680	21/122	0.95 (0.58–1.57)	0.840	52/272	93/530	0.90 (0.62–1.31)	0.593	97/531	48/271	0.98 (0.67–1.43)	0.930
Gender												
Female	106/517	21/78	1.31 (0.78–2.22)	0.310	49/175	78/420	**0.66 (0.45–0.99)**	**0.044**	80/420	47/175	1.41 (0.94–2.11)	0.094
Male	146/718	37/130	1.40 (0.93–2.10)	0.106	76/304	107/544	0.78 (0.56–1.08)	0.133	110/546	73/302	1.21 (0.87–1.68)	0.256
Clinical Stages												
I + II	129/1,235	31/208	1.43 (0.94–2.18)	0.094	68/479	92/964	**0.67 (0.48–0.93)**	**0.017**	95/966	65/477	1.40 (1.00–1.95)	0.051
III + IV	79/1,235	10/208	0.75 (0.38–1.47)	0.395	31/479	58/964	0.94 (0.60–1.48)	0.788	60/966	29/477	0.97 (0.61–1.53)	0.892

The results were in bold if the 95% confidence intervals (CIs) excluded 1, or p values were less than 0.05. AOR, adjusted odds ratio.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.

of hepatocellular carcinoma via the HuR-ETS1-p21/p27 axis.[44] WTAP could also promote the tumorigenesis of cholangiocarcinoma by enhancing the expression of metastasis-related markers matrix metalloproteinase 7 (MMP7) and matrix metalloproteinase 28 (MMP28).[45] However, a study conducted by Sorci et al.[46] showed that loss of function of METTL3 impaired WTAP-mediated cell proliferation. Their data indicated that the reported oncogenic function of WTAP is strictly connected to a functional m^{6}A methylation complex. So far, the role of WTAP in hepatoblastoma has not yet been illustrated.

To date, only one study has been conducted regarding the epidemiologic assessment of *WTAP* gene SNPs. Meng et al.[47] carried out a first case-control study on m^{6}A modification SNPs and colorectal cancer risk. Their study comprised two stages: the discovery stage with 1,150 cases and 1,342 controls and the replication stage with 932 cases and 966 controls. They extensively genotyped 240 SNPs in 20 genes involved in m^{6}A modification. Among them, only one SNP, rs118049207, located in the staphylococcal nuclease and tudor domain containing 1 (*SND1*) gene, predisposes to colorectal cancer risk in the Chinese population. They demonstrated that rs118049207 could regulate mRNA expression of the *SND1* gene and then lead to alteration in the m^{6}A level. However, the rs2842970, rs911846, rs2842973, rs11752345, and rs1535475 in the *WTAP* gene failed to impact colorectal cancer risk.[47]

Given the critical role of m^{6}A modification *WTAP* in cancer, it is essential to investigate the association between *WTAP* gene SNPs and the risk of hepatoblastoma. The current analysis revealed that the rs7766006 T allele was significantly associated with decreased hepatoblastoma risk. We also tested the combined effects of risk genotypes on hepatoblastoma susceptibility. As results, we provided evidence of a correlation between 2 risk genotypes and susceptibility to hepatoblastoma. This significant association was biologically

Table 3. Association between Inferred Haplotypes of the *WTAP* Gene and Hepatoblastoma Risk

Haplotypes[a]	Cases (n = 620) No. (%)	Controls (n = 2,886) No. (%)	Crude OR (95% CI)	p	Adjusted OR[b] (95% CI)	p[b]
GAT	113 (18.23)	530 (18.36)	1.00		1.00	
GAG	180 (29.03)	922 (31.95)	0.92 (0.71–1.19)	0.504	0.92 (0.71–1.19)	0.502
GGT	95 (15.32)	486 (16.84)	0.92 (0.68–1.24)	0.570	0.91 (0.68–1.23)	0.557
GGG	119 (19.19)	423 (14.66)	1.32 (0.99–1.76)	0.059	1.32 (0.99–1.76)	0.059
AAT	30 (4.84)	206 (7.14)	0.68 (0.44–1.05)	0.085	0.69 (0.44–1.06)	0.088
AAG	35 (5.65)	105 (3.64)	**1.56 (1.01–2.41)**	**0.043**	**1.57 (1.02–2.43)**	**0.041**
AGT	4 (0.65)	3 (0.10)	**6.25 (1.38–28.29)**	**0.018**	**6.10 (1.35–27.69)**	**0.019**
AGG	44 (7.10)	211 (7.31)	0.98 (0.67–1.44)	0.910	0.98 (0.67–1.44)	0.912

The results were in bold if the 95% confidence intervals (CIs) excluded 1, or p values were less than 0.05. OR, odds ratio; No., number.
[a]The haplotypes' order was rs9457712, rs1853259, and rs7766006.
[b]Obtained in logistic regression models with adjustment for age and gender.

Table 4. False-Positive Report Probability Analysis for Significant Findings

				Prior Probability				
Genotype	OR (95% CI)	p[a]	Statistical Power[b]	0.25	0.1	0.01	0.001	0.0001
rs1853259 GG versus AG/AA								
<17 months	1.87 (1.21–2.87)	0.005	0.161	**0.077**	0.201	0.734	0.965	0.996
rs7766006 G > T								
GT versus GG	0.70 (0.53–0.92)	0.010	0.714	**0.039**	**0.108**	0.571	0.931	0.993
GT/TT versus GG	0.74 (0.57–0.95)	0.017	0.769	**0.062**	**0.164**	0.684	0.956	0.995
<17 months	0.60 (0.42–0.85)	0.004	0.274	**0.044**	**0.121**	0.603	0.939	0.994
Female	0.66 (0.45–0.99)	0.043	0.483	0.212	0.447	0.899	0.989	0.999
Stage I + II	0.67 (0.48–0.94)	0.019	0.512	**0.100**	0.250	0.786	0.974	0.997
Risk Genotypes								
2 versus 0	1.39 (1.02–1.88)	0.036	0.854	**0.111**	0.272	0.805	0.976	0.998
<17 months	1.64 (1.15–2.32)	0.006	0.318	**0.052**	**0.141**	0.644	0.948	0.995
Haplotypes								
AGT versus GAT	1.56 (1.01–2.41)	0.043	0.491	0.208	0.441	0.897	0.989	0.999
AAG versus GAT	6.25 (1.38–28.29)	0.018	0.037	0.583	0.808	0.979	0.998	1.000

The results were in bold if the false-positive report probability was less than 0.200. 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.

plausible since other m^{6}A modification gene SNPs were also reported to modify cancer risk. In this study, the GG genotype of the rs1853259 was found to associate with hepatoblastoma risk in children under 17 months of age. Compared with the rs7766006 GG genotype, the GT/TT genotype decreased hepatoblastoma risk in children under 17 months of age, female, and patients with stages I + II tumors. After combining the risk genotypes, we observed that patients with 2 protective genotypes were more likely to develop hepatoblastoma in children aged <17 months. We then explore the possible mechanisms for the conferring risk role of rs7766006 G > T. The results from the eQTL analysis indicated that the increased hepatoblastoma risk be linked to the upregulated expression levels of the *WTAP* gene. The rs7766006 T genotype also enhanced the neighboring critical gene *TCP1* mRNA level in the cell-cultured fibroblasts. *TCP1* has been demonstrated to act as an oncogenic gene in several cancers.[48,49] These data shed light on the biological mechanisms of how rs7766006 G > T functions to enhance hepatoblastoma risk.

A major strength of this study is the relatively large sample size. Our study has some limitations, which need to be acknowledged. First, we could not assess the environmental effects on hepatoblastoma risk. Thus, it is debatable whether the observed impact of the *WTAP* gene polymorphisms is modified by these factors. Second, only a single population was adopted in the current study. Studies involving mix ethnicities are needed to evaluate the applicability of the findings to other ethnicities. Third, only three SNPs in the *WTAP* gene were included. More potentially functional *WTAP* SNPs await to be investigated. Fourth, although as the largest sample study performed by far, the sample size of cases was still moderate, and more samples needed to be included in the future. Besides, the biological mechanisms by which the significant SNPs and their host genes regulate hepatoblastoma carcinogenesis remain to be studied.

In summary, our study has illustrated the contributions of *WTAP* gene SNPs to hepatoblastoma risk. To be noted, this is the first multicenter evaluation of the association between *WTAP* gene SNPs and hepatoblastoma susceptibility. Our study underscores the role of *WTAP* gene SNPs in the development of hepatoblastoma. The

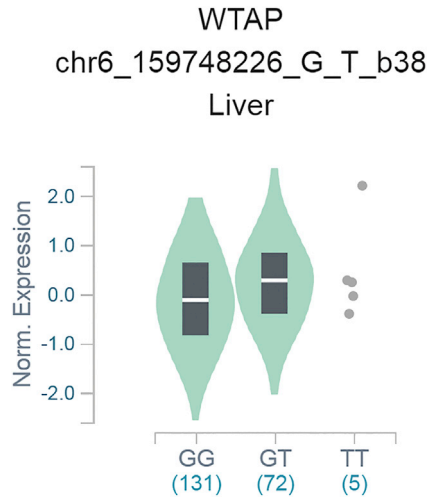

Figure 1. Functional Implication of the *WTAP* Gene rs7766006 Polymorphism in Liver Tissue
The genotype of rs7766006 and expression of the *WTAP* gene in liver tissue were searched based on the public database GTEx portal. $p = 2.90 \times 10^{-5}$.

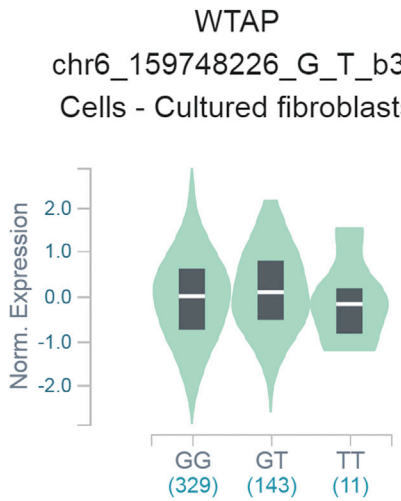

Figure 2. The Functional Prediction of the rs7766006 Polymorphism in Cell-Cultured Fibroblasts

The genotype of rs7766006 and expression of the *WTAP* gene in cell-cultured fibroblasts were searched based on the public database GTEx portal. $p = 7.40 \times 10^{-5}$.

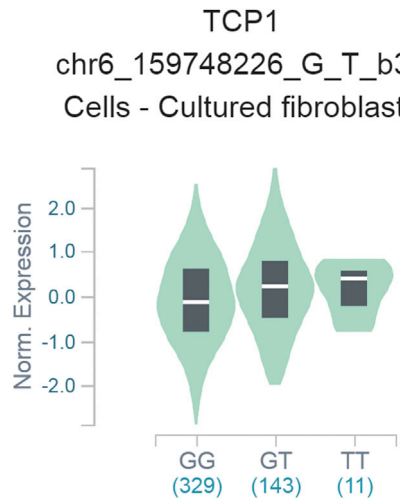

Figure 3. The Functional Prediction of rs7766006 on a Neighboring Gene

Expression correlation between rs7766006 and *TCP1* gene in cell-cultured fibroblasts. $p = 1.80 \times 10^{-7}$.

association of these loci to hepatoblastoma suggests potential biological mechanisms worthy of further investigation.

MATERIALS AND METHODS

Study Subjects

The selection of subjects and collection of specimens have been described previously.[22] The current study included 313 cases and 1,446 controls, enrolled from seven hospitals in China (Guangzhou, Xi'an, Zhengzhou, Changsha, Kunming, Shenyang, and Taiyuan). The patients were all newly diagnosed and pathologically confirmed with hepatoblastoma but without preoperative treatment. Cancer-free controls were recruited from hospital visitors at respective hospitals during the same period. All studies obtained local hospital Institutional Review Board approval and written, informed consent from participants.

Genotyping

Detailed information about SNP selection and genotyping has been described previously.[50] Three SNPs (rs9457712 G > A, rs1853259 A > G, and rs7766006 G > T) were screened out for analysis. Two SNPs (rs9457712 G > A and rs1853259 A > G) affect transcription factor-binding site (TFBS) activity, whereas rs7766006 G > T affects splicing activity. There was no significant linkage disequilibrium (LD) ($R^2 < 0.8$) among these three SNPs of *WTAP* ($R^2 = 0.124$ between rs9457712 G > A and rs1853259 A > G; $R^2 = 0.120$ between rs9457712 G > A and rs7766006 G > T; $R^2 = 0.565$ between rs1853259 A > G and rs7766006 G > T) (Figure S1). Participants' peripheral blood samples were used to extract genomic DNA by the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA). Genotyping for the SNP was performed using the TaqMan assay on an ABI 7900 (Applied Biosystems, Foster City, CA, USA). Negative control samples (water) were used to detect possible reagent and environment contamination in all sequencing batches as well. For quality control, about 10% of randomly selected duplicates were included. All SNPs achieved 100% genotype concordance rates.

Statistical Analysis

The significance of the characteristic differences was analyzed by using the chi-square test or t test as appropriate for comparisons of two groups. A goodness-of-fit χ^2 test was used to evaluate whether an individual SNP in controls complies with the HWE. Hepatoblastoma risk was estimated using ORs and 95% CIs, which were calculated using unconditional multivariate logistic regression. We also conducted stratified analyses to assess whether the effect of *WTAP* gene SNPs is consistent across strata of age, gender, and clinical stages of hepatoblastoma. Logistic regression analyses were adopted to obtain haplotype frequencies and distinct haplotypes, with the adjustment for gender and age.[51,52] FPRP analysis was further conducted to examine whether significant results are just chance findings or noteworthy, as described elsewhere.[53,54] We also performed the eQTL analysis using the GTEx portal web site (https://www.gtexportal.org/home/) to predict potential associations between the SNPs and gene-expression levels.[55] All tests for statistical significance used a two-sided alpha of 0.05. The statistical software SAS, version 10, was used to perform statistical analyses.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omto.2020.06.007.

AUTHOR CONTRIBUTIONS

Z.-J.Z., R.-X.H., Z.C., M.W., H.X., and J.H. designed and performed the study and wrote the manuscript. Z.Y., J. Zhang, Y.L., L.L., S.L.,

Y.X., and J.H. collected the samples and information. J. Zhu and J.H. participated in analyzing data. Z.-J.Z., H.X., and J.H. coordinated the study over the entire time. All authors reviewed the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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