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Research Paper

Association between the SNPs of the *TOB1* gene and gastric cancer risk in the Chinese Han population of northeast China

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Abstract

The *TOB1* (ErbB-2,1) gene is an anti-proliferative factor that has the potential to regulate cell growth and encodes a member of the transducer of erbB-2/B-cell translocation gene protein. The association between the polymorphisms of the *TOB1* gene and gastric cancer (GC) risk is still unclear. In this study, 506 GC cases and 548 healthy controls (HCs) were collected to evaluate the association between the eleven SNPs (rs35220381, rs12950561, rs7221352, rs61482741, rs9303568, rs34700818, rs12949115, rs9903822, rs12601477, rs11656976 and rs4626) of the *TOB1* gene and GC risk in the population of northeast China. The results showed that there were significant associations of haplotype GCCTTGC, haplotype ATCTTGG, and haplotype GCCACGC with GC risk ($P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively). The association between rs12601477 GA+AA genotypes and GC risk was significant among individuals older than 58 (adjusted OR=1.53, 95% CI=1.05-2.22, $P< 0.05$). The association between rs4626 AG+GG genotypes and GC risk was significant among individuals older than 58 (adjusted OR=1.54, 95% CI = 1.03-2.28, $P<0.05$). The rs34700818 CT+TT genotypes were associated with a significantly increased risk of T3-T4 (CT+TT vs CC, adjusted OR=1.71, 95% CI= 1.01-2.88, $P<0.05$) and TNM stage II (CT+TT vs CC, adjusted OR=2.40, 95% CI =1.27-4.52, $P<0.01$). The rs61482741 CG+GG genotypes were also associated with a significantly increased risk of T3-T4 (CG+GG vs CC, adjusted OR=1.71, 95% CI = 1.01-2.88, $P<0.05$) and TNM stage II (CG+GG vs CC, adjusted OR=2.40, 95% CI=1.27-4.52, $P<0.01$). The results suggest that four SNPs (rs12601477, rs4626, rs34700818 and rs61482741) of the *TOB1* gene play an important role in the occurrence and development of GC in the Chinese Han population of northeast China.

Key words: gastric cancer; *TOB-1*; single nucleotide polymorphism; genetic risk

Introduction

Gastric cancer (GC) is the fifth most common neoplasia and the third leading cause of cancer-related death worldwide [1]. There were an estimated 951,000 new cases in the world in 2012, and approximately half of the total GC cases were in China [2]. Despite the overall decline in adverse outcomes with the advances in diagnosis and treatment in most of the Western world, GC remains a serious fatal disease and has a poor prognosis throughout Asia, especially in China [3]. GC as a heterogeneous disease shows distinct clinical, epidemiological, and molecular features among tumors arising from the cardia or non-cardia stomach and among the intestinal and diffuse histological

subtypes [4, 5]. Additionally, GC is a multi-step and multi-factorial disease that is influenced by environmental factors, microbial infections, and the host genetic background [6, 7].

The *TOB1* (transducer of ErbB2,1) gene is a member of the TOB/B cell translocation BTG family, which includes BTG1, BTG2/TIS21/PC3, BTG3/ANA, BTG4/PC3B, TOB1/TOB, and TOB2 [8]. The *TOB1* gene is located on chromosome 17q21 and codes for a 45-kDa protein, which was first discovered in the 1990s [9]. As a tumor suppressor, the effect of the *TOB1* gene involves many aspects, including anti-proliferation, inhibition of transcription, and the reduction of the migration and invasion of tumor cells in thyroid [10], breast [11], and lung [12] cancers.

We recently identified several allelic deletions on chromosomes 17 and 18 in 45 primary GCs using microsatellite markers for the loss of heterozygosity (LOH). *TOB1* lies in one of these regions (17q21.3-22) on the long arm of chromosome 17 [13, 14]. Then, we demonstrated that the down-regulation of *TOB1* expression and the accumulation of phosphorylated *TOB1* promoted carcinogenesis in four GC cell lines and tissue specimens from 97 patients with primary GC [15]. Furthermore, we identified that decreased *TOB1* expression and increased phosphorylation of nuclear *TOB1* were associated with a malignant tumor phenotype and poor survival in 341 primary GC patients [16].

Recently, increasing numbers of studies have identified that genetic variation plays an important role in the development of most diseases, especially in tumors [17]. Single nucleotide polymorphisms (SNPs), as the most common form of genetic variation, play an important role in the development of GC [18, 19]. Increasingly more studies have been performed to investigate the association between tumor suppressor gene polymorphisms and GC risk [18, 20]. A GWAS (genome wide association study) revealed that two SNPs in the PSCA gene were associated with an increased diffuse-type GC risk in a Korean and Japanese population [21]. A meta-analysis indicated that in the P53 codon, 72 polymorphisms might be associated with GC among Asians [22]. However, the association of the SNPs in the TOB1 gene with the risk in malignant tumors (including GC) has not been reported.

TOB1, as a tumor suppressor, encodes a member of the transducer of erbB-2/B-cell translocation gene protein and has the potential to regulate cell growth. However, it is not clear what role the *TOB1* gene polymorphisms play in GC risk. Here, we investigated the association between some SNPs in the *TOB1* gene and GC risk in a set of 506 GC patients and 548 healthy controls (HCs). Our results suggested that SNPs (rs12601477, rs4626, rs34700818, and rs61482741) in the *TOB1* gene are important markers for GC risk in the Chinese Han population.

Material and Methods

Study population

A total of 506 unrelated Han Chinese primary GC patients were recruited from The Tumor Hospital Affiliated to Harbin Medical University between January 2015 and June 2016. A total of 548 age- and sex-matched HCs were recruited from The Second Affiliated Hospital to Harbin Medical University during the same period. This study was approved by the ethic committees at local hospitals.

Each participant was interviewed face-to-face by trained interviewers using a standardized questionnaire. The data, including age, gender, family history, native origin, pathological diagnosis, smoking status and alcohol consumption, were collected. Individuals who smoked at least once a day for more than a year were defined as smokers, and the others were defined as non-smokers. Those who consumed alcohol at least once a week for more than a year were defined as drinkers, while the remaining were non-drinkers. The clinical data and demographics of the GC patients and the HCs are summarized in Table 1.

Written informed consent was obtained from each participant. With the permission of the subjects, peripheral venous blood (5 ml) was collected and stored at -80°C in EDTA tubes for DNA extraction.

SNP selection

The potentially functional SNPs were selected using the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp) and SNPinfo (http://snpinfo.niehs.nih.gov/snpinfo/snpfuc.htm). The criterion applied on the minor allele frequency (MAF) in the NCBI was greater than 0.05 in the Chinese Han population. Ultimately, thirteen SNPs (rs78420930, rs35220381, rs12950561, rs7221352, rs61482741, rs9303568, rs34700818, rs12949115, rs9903822, rs12601477, rs11656976, rs9898809 and rs4626) were selected in our study.

Genotyping

Genomic DNA was extracted from the blood samples using the Qiagen Blood DNA Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. The LDR and SNaPshot methods were used to acquire the genotypes of all the SNPs. Genotyping was repeated on a random 10% of the samples, and the results were 100% concordant. Ultimately, eleven SNPs (rs35220381, rs12950561, rs7221352, rs61482741, rs9303568, rs34700818,

rs12949115, rs9903822, rs12601477, rs11656976 and rs4626) were successfully genotyped in 1054 subjects (506 GCs and 548 HCs) and were available for analysis. Two SNPs could not be used to design primers for PCR amplification because of the high GC content.

Table 1. Clinical and demographic characteristics of cases and controls

Variables	Case, n (%)	Control, n (%)	*P*[a]
All subjects	506(100.0)	548(100.0)	
Age	23-75	25-87	0.243
Mean[b]	59.1±10.55	58.3±11.61	
≤50	107(21.2)	130(23.7)	
51-60	156(30.8)	166(30.3)	
61-70	169(33.4)	172(31.4)	
≥71	74 (14. 6)	80 (14. 6)	
Gender			0.363
Male	371(73.3)	388(70.8)	
Female	135(26.7)	160(29.2)	
Smoking status			**<0.0001**
Never	240(47.4)	423(77.2)	
Ever	266(52.6)	125(22.8)	
Drinking status			**<0.0001**
No	306(60.5)	419(76.5)	
Yes	200(39.5)	129(23.5)	
Pack-years			**<0.0001**
0	240(47.4)	423(77.2)	
≤25	78 (15.4)	26 (4.7)	
>25	188(37.2)	99 (18.1)	
Neoplasia location			
GCA	70(13.8)	—	
NGCA	435(86.0)	—	
Else	1 (0.2)	—	
Lauren's classification			
Intestinal	203(40.1)	—	
Diffuse	71 (14.1)	—	
Mixed	81 (16.0)	—	
Else	151(29.8)	—	
TNM stage			
I	87 (17.2)	—	
II	156(30.8)	—	
III	128(25.3)	—	
IV	66 (13.0)	—	
Else	69 (13.6)	—	
Family history of cancer			
none	401(79.3)	—	
Gastric cancer	42 (8.3)	—	
Other cancer	63 (12.5)	—	

Notes: GCA, gastric cardia adenocarcinoma; NGCA, non-gastric-cardia ademocarcinoma

[a]Two-sided χ^2 test for distributions between cases and controls [b]Data are mean ± SD

The bold values indicate statistically significant data

Statistical analysis

Continuous variables with a normal distribution were described as the mean ± standard deviation and were compared using a Student's t-test. Discrete variables were described as the frequency (percentage) and were compared using the Chi-square (χ^2) test. The genotype frequencies for all the *TOB1* gene polymorphisms of the controls were tested for Hardy-Weinberg equilibrium by using a Chi-square test. Associations of the genotypes and alleles with the risk of GC were estimated by the odds ratios (ORs) and 95% confidence intervals (CIs). Linkage disequilibrium (LD) and haplotype analyses were performed with Haploview 4.2 software (http://sourceforge.net/projects/haploview/). P values and ORs with 95% CIs were calculated using a logistic regression analysis adjusted for age, gender, smoking status, pack-years, and drinking status. All the statistical analyses were performed using the SAS 9.3 software. All P values in the study were two-sided, and $P<0.05$ was considered statistically significant.

Results

Population characteristics

A total of 1054 participants (506 GCs and 548 HCs) were enrolled in this study. All the characteristics of the subjects are shown in Table 1. No significant difference between the GC and HC subjects regarding age and gender ($P=0.243$ and $P=0.363$) were found, which indicated that the frequency matching was adequate. The mean age was 59.1 (59.1±10.55 years) for the patients and 58.3 (58.3±11.61 years) for the controls. However, there was a significant difference ($P<0.0001$) between the cases and controls regarding smoking status, drinking status, and pack-years. Of the GCs, 70 (13.8%) cases were diagnosed with gastric cardia adenocarcinoma, while 435 (86.0%) cases were diagnosed with non-gastric cardia adenocarcinoma, and 1 (0.2%) was unclear. In term of stage, 87 (17.2%), 156 (30.8%), 128 (25.3%), 66 (13.0%), and 69 (13.6%) cases were classified as TNM stages I, II, III, IV, and else, respectively, according to the 7th Edition of the American Joint Committee on Cancer (AJCC) [23].

The numbers of participants with no family history of cancer, a history of GC, and a history of other cancers were 401 (79.2%), 42 (8.3%), and 63 (12.5%), respectively.

Association between polymorphisms of the *TOB1* gene and the risk of GC

The genotype distributions of the eleven SNPs among the cases and controls and their associations with GC risk are summarized in Supplementary Table 1. The genotype frequencies of all the SNPs of the controls were in accordance with Hardy-Weinberg equilibrium ($P>0.05$). All the allele frequencies were not significantly different in the case and control groups. Variables including age, gender, smoking, drinking, and pack-years were adjusted for in the subsequent logistic regression analyses. The result showed that none of the eleven SNPs were associated with GC risk in the homozygotes or heterozygotes after adjusting for age, gender, smoking status, pack-years and drinking status ($P> 0.05$).

Table 2. The frequencies of haplotypes of the *TOB1* gene in cases and controls

Haplotype	Frequency	Haplotype frequencies in GC	Haplotype frequencies in HC	c^2	*P*
Block1					
ACC	0.566	0.553	0.578	1.353	0.245
GCA	0.254	0.257	0.251	0.087	0.768
GCC	0.110	0.117	0.104	0.980	0.322
GTC	0.063	0.066	0.061	0.184	0.668
Block2					
GCCTTGC	0.618	0.594	0.641	4.861	**0.028**
ATCACGG	0.236	0.228	0.244	0.772	0.380
ACTACAC	0.061	0.058	0.063	0.252	0.616
ACCACAC	0.035	0.039	0.032	0.792	0.373
ATCTTGG	0.016	0.031	0.002	27.232	**<0.001**
GCCACGC	0.011	0.022	0.001	20.638	**<0.001**

Notes: The bold values indicate statistically significant data

Haplotype analysis and GC risk

Strong LDs for the SNPs of the *TOB1* gene were identified by the Haploview software (Figure 1). Two blocks in the *TOB1* gene were found, with four haplotypes in block 1 and six haplotypes in block 2. The associations between the frequencies of the haplotypes and GC risk are shown in Table 2. The most common haplotype of block 1 was determined as ACC (0.566), followed by GCA (0.254), GCC (0.110), and GTC (0.063). No association between the haplotypes of block 1 and GC risk was observed. The most common haplotype of block 2 was determined as GCCTTGC (0.618), followed by ATCACGG (0.236), ACTACAC (0.061), ACCACAC (0.035), ATCTTGG (0.016), and GCCACGC (0.011). The results showed that there were significant associations of haplotype GCCTTGC, haplotype ATCTTGG, and haplotype GCCACGC with GC risk (*P*= 0.028, *P*< 0.001, and *P*<0.001, respectively).

Stratified analysis and GC risk

We conducted stratified analyses for all the SNPs according to age, gender, smoking status, pack-years, and drinking status, which have potential influences on the genetic effect in the 506 cases and 548 controls of the 1054 participants (Table 3 and Tables S2-S6). As shown in Table 3, for SNP rs12601477, the GA+AA genotypes were associated with a significantly increased risk of GC among individuals older than 58 (GA+AA vs GG, adjusted OR=1.53, 95% CI = 1.05-2.22, *P*=0.025). The rs4626 AG+GG genotypes were also associated with a significantly increased risk of GC among individuals older than 58 (AG+GG vs AA, adjusted OR=1.54, 95% CI = 1.03-2.28, *P*=0.033). No significant association was found between other genotypes of the SNPs and the risk of GC in the stratified analyses by age, gender, smoking status, pack-years, and drinking status.

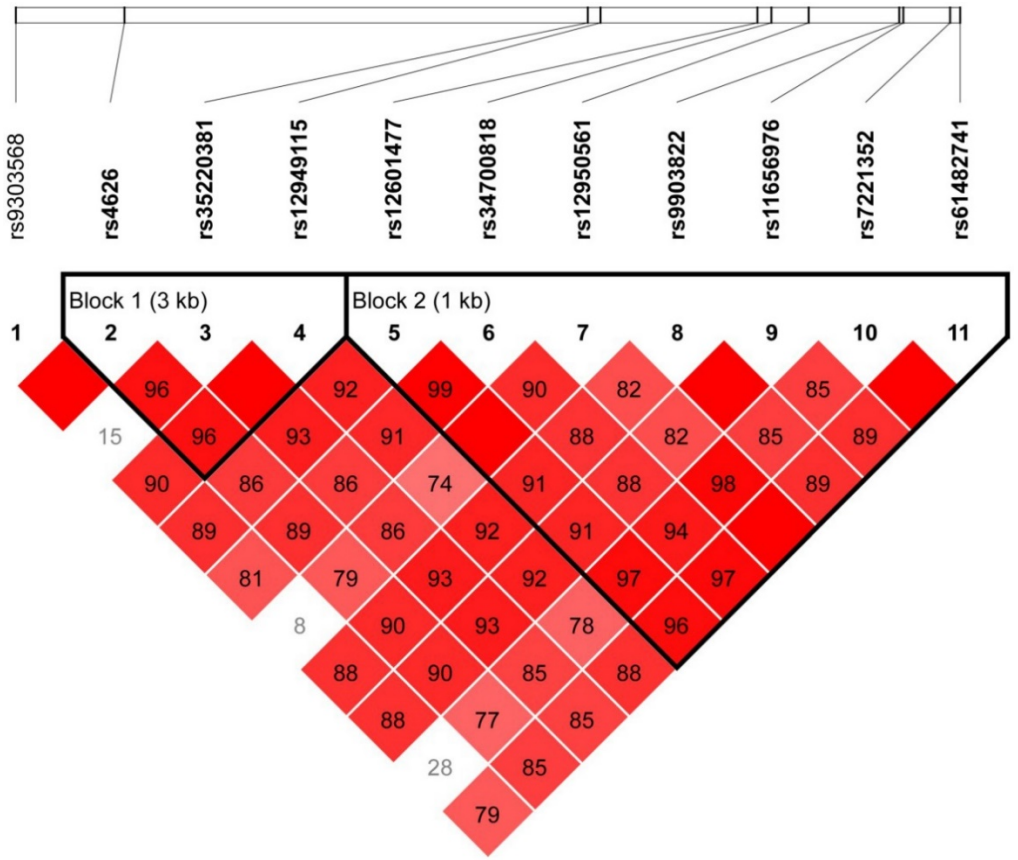

Figure 1. Linkage disequilibrium (LD) map covering *TOB1* gene

Table 3. Stratified analyses for *TOB1* gene rs12601477 and rs4626 genotypes in cases and controls

Variables	rs12601477 (cases/controls)		OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*[a]	rs4626 (cases/controls)		OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*[a]
	GA+AA	GG					AG+GG	AA				
Age												
≤58	138/158	93/107	1.00 (0.70-1.44)	0.979	0.98 (0.67-1.43)	0.913	149/179	82/86	0.88 (0.60-1.27)	0.474	0.88 (0.59-1.30)	0.514
>58	176/153	99/130	**1.51 (1.08-2.12)**	**0.017**	**1.53(1.05-2.22)**	**0.025**	199/182	76/101	**1.45 (1.01-2.08)**	**0.041**	**1.54 (1.03-2.28)**	**0.033**
Gender												
Male	229/216	142/172	1.28 (0.96-1.72)	0.091	1.22 (0.89-1.66)	0.214	253/252	118/136	1.16 (0.86-1.56)	0.344	1.15 (0.84-1.59)	0.385
Female	85/95	50/65	1.16 (0.73-1.86)	0.529	1.51 (0.91-2.53)	0.114	95/109	40/51	1.11 (0.68-1.83)	0.678	1.42(0.82-2.46)	0.207
Smoking status												
Nonsmoker	154/239	86/184	1.38 (0.99-1.91)	0.054	1.35 (0.97-1.87)	0.077	172/280	68/143	1.29 (0.91-1.83)	0.146	1.26 (0.89-1.78)	0.201
Smoker	160/72	106/53	1.11 (0.72-1.71)	0.632	1.18(0.75-1.84)	0.474	176/81	90/44	1.06 (0.68-1.66)	0.791	1.10 (0.69-1.76)	0.674
Pack-years												
0	154/239	86/184	1.38 (0.99-1.91)	0.054	1.35 (0.97-1.87)	0.077	172/280	68/143	1.29 (0.91-1.83)	0.146	1.26 (0.89-1.78)	0.201
≤25	48/17	30/8	0.75 (0.29-1.96)	0.561	0.69 (0.25-1.92)	0.477	49/19	29/6	0.53 (0.19-1.49)	0.230	0.42(0.14-1.25)	0.119
>25	112/55	76/45	1.21 (0.74-1.97)	0.454	1.31 (0.79-2.17)	0.292	127/62	61/38	1.28 (0.77-2.12)	0.345	1.38 (0.82-2.32)	0.231
Drinking status												
Nondrinker	185/240	121/179	1.14 (0.84-1.54)	0.391	1.16 (0.84-1.59)	0.373	212/279	94/140	1.13 (0.82-1.55)	0.4436	1.13(0.81-1.58)	0.476
Drinker	129/71	71/58	1.48(0.94-2.33)	0.087	1.49 (0.92-2.40)	0.102	136/82	64/47	1.22 (0.76-1.94)	0.4065	1.26(0.77-2.06)	0.365

Notes: CI, confidence interval; OR, odd ratio

[a]Adjusted for age age, gender, smoking status, pack-years and drinking status

The bold values indicate statistically significant data

Table 4. Association between *TOB1* (rs34700818 and rs61482741) genotypes and clinicopathologic characteristics of GC

Variables	rs34700818				rs61482741			
	CT+TT (n)	CC (n)	Adjusted OR (95% CI)[a]	*P*	CG+GG (n)	CC (n)	Adjusted OR (95%CI)[a]	*P*
Family history of cancer								
none	104	114	1		104	114	1	
Gastric cancer	11	13	0.92 (0.39-2.16)	0.844	11	13	0.92 (0.39-2.16)	0.844
Other cancer	15	17	1.03 (0.48-2.18)	0.948	15	17	1.03 (0.48-2.18)	0.948
Tumor size(cm)								
<5	71	90	1		71	90	1	
≥ 5	59	54	1.41 (0.86-2.31)	0.176	59	54	1.41 (0.86-2.31)	0.176
Neoplasia location								
Non-cardia	117	121	1		117	121	1	
Cardia	13	23	0.56 (0.27-1.18)	0.125	13	23	0.56 (0.27-1.18)	0.125
Invasion depth								
T1-T2	35	55	1		35	55	1	
T3-T4	95	89	**1.71 (1.01-2.88)**	**0.046**	95	89	**1.71 (1.01-2.88)**	**0.046**
Lymph metastasis								
N0	51	62	1		51	62	1	
N1/N2/N3	79	82	1.06 (0.64-1.75)	0.817	79	82	1.06 (0.64-1.75)	0.817
TNM stage								
I	23	46	1		23	46	1	
II	67	54	**2.40 (1.27-4.52)**	**0.007**	67	54	**2.40(1.27-4.52)**	**0.007**
III	40	44	1.73 (0.88-3.43)	0.114	40	44	1.73 (0.88-3.43)	0.114
Lauren's classification								
Intestinal	95	108	1		95	108	1	
Diffuse	35	36	1.12 (0.62-2.03)	0.714	35	36	1.12 (0.62-2.03)	0.714

Notes: CI, confidence interval; OR, odd ratio

[a]Adjusted for age, gender, smoking status, pack-years and drinking status

The bold values indicate statistically significant data

We also conducted stratified analyses based on a family history of cancer, tumor size, neoplasia location, depth of invasion, lymph metastasis, TNM stage and Lauren's classification in 274 patients (Table 4 and Tables S7-S11). As shown in Table 4, the rs34700818 CT+TT genotypes were associated with a significant increase in T3-T4 compared with T1-T2 (CT+TT vs CC adjusted OR=1.71, 95% CI=1.01-2.88, *P*=0.046) and with a significant increase in TNM stage II compared with stage I (adjusted OR=2.40, 95% CI=1.27-4.52, *P*=0.007). The rs61482741 CG+GG genotypes were also associated with a significant increase in T3-T4 compared with T1-T2 (CG+GG vs CC, adjusted OR=1.71, 95% CI = 1.01-2.88, *P*=0.046) and with a significant increase in TNM stage II compared with stage I (CG+GG vs CC, adjusted OR=2.40, 95% CI = 1.27-4.52, *P*=0.007). No association was found between other genotypes of the SNPs and the clinicopathological characteristics of GC.

Discussion

We previously identified that three overlapping regions (R1-R3) highlighted the association between

the LOH on chromosome 17 and GC pathogenesis [13]. Afterward, we narrowed down these intervals using high-density genome scanning and defined five smaller overlapping subregions of LOH (SR1-SR5) in the GC samples. Ultimately, we focused on the *TOB1* gene in SR3 (17q21.33), which has not been investigated in GC. Furthermore, our studies demonstrated the down-regulation of *TOB1* expression in 75% of primary GCs and the accumulation of phosphorylated *TOB1* in GC cells [15]. Recently, we identified that decreased *TOB1* expression and increased nuclear phosphorylated *TOB1* were associated with aggressive tumor behavior and a poor prognosis in intestinal type GC. Additionally, *TOB1* nuclear retention is critical for its anti-proliferative activity *in vitro* [16].

Multiple factors, including *Helicobacter pylori* (Hp) infection [22], nutrition deficiency, a high salt diet, and the chemical carcinogens in tobacco [24, 25], may play an important role, although the etiology and pathogenesis of GC is still uncertain. Furthermore, even if exposed to the same exogenous environmental factors, only a small fraction of people will develop GC, which implies that endogenous genetic variation may also contribute to the individual susceptibility to GC. Recent studies suggest that SNPs may be related to gastric tumorigenesis [19]. Huang C et al. demonstrated that the CT+TT genotypes of the DACT1 rs863091 polymorphism were significantly associated with a decreased risk of GC in the Chinese Han population, especially in younger individuals and males [18]. DACT1 is a tumor suppressor gene that suppresses tumorigenesis in GC by inhibiting the NF-κB signaling pathway [26]. NF-κB might participate in β-catenin-mediated target gene expression and eventually results in enhanced tumor growth [27]. *TOB1* is a functional anti-oncogene that mainly induces apoptosis and inhibits proliferation, migration and invasion via the activation of Smad4 and the suppression of the β-catenin-mediated signaling pathways in GC [28].

In the present study, we investigated the associations between the polymorphisms of the *TOB1* gene and GC risk in the Chinese Han population. We compared single allele frequencies and a genetic model of the polymorphisms in case-control groups, and no significant differences were found. However, we found that several SNPs of the *TOB1* gene were in strong linkage disequilibrium, and haplotype GCCTTGC, haplotype ATCTTGG, and haplotype GCCACGC were significantly associated with GC risk. Moreover, GCCTTGC was the most common haplotype of the *TOB1* gene, with a frequency up to 61.8%. Several studies suggest that haplotypes are associated with tumors, such as in TP53, for which the haplotype CCA decreases the risk for GC in a Spanish population [29, 30]. The polymorphisms included in block 2 were upstream variants of the *TOB1* gene, and for the first time, they were associated with cancer risk. We plan to identify their effects in gastric tumorigenesis in future studies.

We also found that the rs12601477 GA+AA genotypes and the rs4626 AG+GG genotypes were associated with a significantly increased risk of GC among individuals older than 58. This difference may be related to the weaker or disordered immune system in older individuals [31]. *TOB1* is expressed in several cell types. It associates with Smad2 and Smad4 DNA binding and Smad-dependent transcription in T lymphocytes [32]. Increasingly more reports indicate that the *TOB1* gene is associated with human immune-related disorders [33]. Low levels of *TOB1* may promote an aberrant immune response and affect disease progression [34].

The rs34700818 CT+TT genotypes and the rs61482741 CG+GG genotypes were associated with a significant increase in T3-T4 and TNM stage II. The function of *TOB1* involves anti-proliferation, inhibition of transcription and reduction of cancer cell migration, invasion, and metastasis [12]. Gene polymorphisms produced by the replacement, insertion, and loss of a base, which lead to a change in the sequence of the nucleotide, affect the transcription and translation process and ultimately affect the expression of proteins. A GWAS identified that several SNPs are significantly associated with GC. Two GWASs demonstrated that the genetic variant loci in PLCE1 at 10q23 and, for non-cardia GC, at 3q13.31 and 5p13.1 increase the risk of tumors in the stomach [35, 36]. A SNP (rs41274221) in miR-25 regulates the expression of *TOB1* by binding with its 3'-UTR region in GC. The mutant genotype promotes cell proliferation and suppresses apoptosis by changing the expression of *TOB1* in GC [37].

To date, an increasing number of researches focus on the role of *TOB1* in gastric cancerogenesis, which including the localization and expression of the gene[13-15], the mechanisms of inactivation, tumor suppressor involving the participating pathways, and its effect in the cell cycle, etc. [16, 28, 38]. A new study reported that *TOB1* gene was contribute to estrogen-independent breast cancer and the interaction effects between *TOB1* gene with AKT/mTOR survival signaling [39]. All of the above studies were carried out in cancer tissue or cell lines. Our study is an expansion of the previous work, and it is the first time to investigate the association between *TOB1* gene polymorphism and GC susceptibility with molecular markers in peripheral blood. Our results showed that the genotypes of the

two SNPs (rs34700818 and rs61482741) have significant association with the increased invasion and severity, which suggesting that they impair the inhibition of *TOB1*. The study provides a direction of predicting the prognosis of the progress of GC and developing some targeting drugs based on these two SNPs. Next, we will continue to enlarge the sample size and to perform functional verification.

There are several limitations in this study, which should be considered. First, partial missing clinical information of the cases, such as data on the TNM stage and Lauren's classification, prevented further analysis. Second, Hp infection is not only an independent but also an important risk factor of GC. The examination of HP infection is not yet a routine examination item. Thus, we did not collect enough information on HP infection for all the subjects, especially for the controls. Third, although our sample size was relatively large, the samples were dispersed in the subgroup analysis and the multilayer analysis. Hence, we will continue to increase the number of GC samples in the next study. Despite these limitations, this is the first study to examine the role of *TOB1* gene polymorphisms in the susceptibility to GC. Our findings provide a novel clue for the associations between the SNPs of the *TOB1* gene and GC risk in the population of northeast China. Further studies should focus on the effect of the two polymorphisms in the *TOB1* gene on gastric tumorigenesis.

In conclusion these results indicated, for the first time, that the four SNPs (rs12601477, rs4626, rs34700818 and rs61482741) of the *TOB1* gene are related with GC risk in the Chinese Han population of northeast China. And there are significant association between three haplotypes and GC risk.

Supplementary Material

Supplementary tables.
http://www.jcancer.org/v09p1371s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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