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RESEARCH

Association of Romo1 Gene Genetic Polymorphisms with Risk of Gastric Cancer in Northwestern Chinese Population

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Abstract Increased expression of reactive oxygen species modulator 1 protein-triggered reactive oxygen species production was reported in the mitochondria of various cancer cell lines. To date there is no report on association between Romo1 gene polymorphisms and gastric cancer risk. To investigate the relationship between Romo1 gene polymorphisms and GC risk, we conducted a case-control study in a population from northwest China (358 GC patients and 412 healthy controls). The genotypes of two SNPs were determined with PCR–denaturing high-performance liquid chromatography and direct DNA sequencing. We found that the genotype and allele distributions of two polymorphisms were significantly different in GC patients compared with controls, When the wild type of two loci were served as the reference group, respectively, significantly increased risk for gastric cancer were associated with rs6060566 TC genotype (Adjusted OR= 1.525, 95 % CI =1.126–2.138), rs6060567 GC genotype (Adjusted OR=1.641, 95 % CI =1.238–2.291) and CC genotype (Adjusted OR=1.594, 95 % CI =1.102–2.973). This effect was more pronounced in patients with smoking, alcohol consumption, H.pylori infection,and male patients subgroups. Haplotypes analysis of two genetic variants showed that the most common haplotype TG displayed the strongest evidence of association with GC (corrected $P=9.30\times10^{-5}$), and was associated with protection against GC (OR=0.584). Whereas the CC haplotypes had significant correlation with GC risk (OR=1.732). These findings suggested genetic polymorphisms of Romo1 gene were associated with significant risk of GC in Northwestern Chinese population, which is strengthened by alcohol use, smoking, H.pylori infection or male patients.

Keywords Gastric cancer · Romo1 gene · Single nucleotide polymorphisms · DHPLC

Abbreviations

Romo1	Reactive oxygen species modulator 1
ROS	Reactive oxygen species
GC	Gastric cancer
DHPLC	Denaturing high performance liquid chromatography

Introduction

Gastric cancer (GC) is the second leading cause of cancer-related death in the world. Almost two thirds of the cases occur in developing countries and 42 % in China alone [1]. The mortality of GC is higher in north-western region than other places in China [2]. Gastric cancer is a heterogeneous disease with multistage and multifactorial process which involves irritant environmental and genetic factors [3, 4]. Recently, involvement of mitochondria in free radical generation

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and apoptosis has stimulated interest in examining the potential role of mitochondria in cancer [5, 6].

An imbalance in reactive oxygen species (ROS) production alters the intracellular redox homeostasis, triggers DNA damage, and contributes to cancer development and progression [7]. Previous reports demonstrated a higher level of ROS observed in cancer cells as well as in cancer patients, which can cause oxidative damage [8]. The mitochondrial inner-membrane protein reactive oxygen species modulator 1 (Romo1), which induces ROS generation, was originally identified in tumor cells and increased Romo1 expression was found in most tumor cell lines [9]. Romo1-derived ROS was indispensable for both normal and cancer cell proliferation, which suggested that Romo1-induced ROS may play an important role in redox signaling in cancer cells [10, 11]. Romo1 gene is located in chromosome 20q11.22 [12]. SNP rs6060566 and rs6060567 are located in 2-intron of Romo1 gene. To date there is no report on association between Romo1 gene polymorphisms and GC risk.

Increasing evidence supports the relationship between variability of genes related mitochondrion and differences in energy metabolism, which may have pathophenotypic consequences [13–15]. The aim of present study was to assess whether the polymorphisms of Romo1 gene are associated with the risk of gastric cancer in a Chinese population. Furthermore, we also explored the interaction between the polymorphisms and environmental factors (Gender differences, smoking status, alcohol consumption, and H.pylori infection) involved in gastric carcinogenesis.

Materials and Methods

Study Subjects

Lanzhou University Ethics Committee granted approval of this case-control study, each participant signed an informed consent form after receiving a detailed verbal explanation of the study. A total of 358 GC patients and 412 healthy controls were consecutively recruited between January 2008 and November 2009 from four hospitals in Wuwei city of Gansu province in Northwestern of China. All of the GC patients were histopathologically confirmed in the patients during surgery at four hospitals, none of the patients received any other treatment such as radiation therapy or chemotherapy before operation. The controls were cancer-free healthy subjects matched with age, gender, area of residence and ethnicity from checking-up in the same hospital, yet not with a family history of GC. All subjects in the case and control groups were ethnic Han Chinese from northwest China. The participants were face-to-face interviewed by specially trained investigators, using a detailed questionnaire; Questions included demographic characteristics (e.g., age, gender, social economics, educational level and job etc.), current and previous smoking behavior in their lifetime, personal medical and psychological conditions, and family history of cancer. Participants were classified into three groups according to their smoking status. Never-smokers were those who had never smoked in their lifetime. Former smokers (ex-smoker) were defined as persons who had previously smoked more than one cigarette each day but had quit smoking for more than 1 year. Whereas current-smokers were defined as individuals who reported that they were still smoking at the time of their gastric cancer diagnosis. And those who consumed three or more alcoholic drinks a week for over 6 months were considered to be drinkers.

The concentrations of serum immunoglobulin (Ig) G antibodies to H. pylori were detected by ELISA (H. pylori-IgG kit; Biohit Co., Ltd., Helsinki, Finland). An individual whose antibody titer defined by optical density (OD) values according to the manufacturer's protocol, was higher than the cut off value of 42EIU, were regarded as positive for H. Pylori infection.

DNA Extraction

Genomic DNA was extracted from EDTA anticoagulated peripheral blood by proteinase K digestion according to a standard proteinase K digestion and phenol/chloroform extraction procedures. The clots and sera were stored immediately at −20 °C, and then moved into a freezer at −70 °C until they were used for genotype testing and other clinical parameters measuring.

SNPs Selection and PCR Amplification

Patients and control subjects were genotyped for two Single nucleotide Polymorphisms (SNPs) in the intron 2 (rs6060566, rs6060567) of Romo1 gene. Primers for the two loci were as follow (rs6060566 and rs6060567 are designed in the same fragment, Forward 5′-actcgttcagcggttttact-3′ and Reverse 5′-ctggtggatgtcgtattaac-3′). The fragment (421 bp) was amplified under the standard PCR conditions (annealing temperature= 58.0 °C). PCR products were visualized by 1.5~2.0 % agarose gels electrophoresis on stained with ethidium bromide (EB).

Genotypes Determined by DHPLC and DNA Directly Sequencing

The Genotypes of those two polymorphisms were conducted by WAVE 3500 apparatus (Transgenomic Inc., Omaha, USA). Denatured and re-annealed PCR products (6 μl; heated at 95 °C for 5 min and cooled to 56 °C at a rate of 0.1 °C/s) were directly injected to the plate. Oven temperature for optimal heteroduplex separation under partial DNA denaturation

was determined for PCR fragment using DHPLC Melt Program (Oven temperature=55.8 °C). The gradient for the elution of fragment (buffer A: 0.1 M triethylammonium acetate; buffer B: 0.1 M triethylammonium acetate, 25 % acetonitrile) was regulated automatically by the Navigator software. Samples in question were mixed with sequence-known wild-type sample (ratio 1 : 1) to screen corresponding mutations, and their elution profiles were compared with standard reference profile. Data was collected and analyzed by the WAVEMAKER 4.1 software (Transgenomic Inc., Omaha, USA). The results were further confirmed by direct-sequencing (percentage of sequenced samples >10 %) and DHPLC reanalysis by mixing mutation samples. The results of DNA sequencing were compared with the reference Sequence (NM_080748.2) using Mutation Surveyor Version 2.2 (Soft Genetics LLC,State College, USA).

Statistical Analysis

Allele and genotype frequencies of two SNPs were obtained by direct counting. For allele comparisons, the chi-square test was used. Odds ratios (ORs) and 95 % confidence intervals (95 % CIs) were calculated according to the Woolf method. Between-group differences in continuous variables were evaluated using the Student's t-test. Unconditional logistic regression was used to analyze the association between genotypes and GC risk, adjusted for gender, age and other environmental factors (smoking status, alcohol consumption, etc.). The comparisons were also conducted according to the different group histological grade of GC [16]. Hardy-Weinberg Equilibrium (HWE) was calculated by the Chi-square test for goodness of fit in both patient and control groups. If necessary, were performed to assess the difference in genotype frequencies in difference gender and other environmental factors. Statistical analysis was performed with the SPSS version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). All P value was two-tailed, and P values less than 0.05 were considered statistically significant. Multiple testing was corrected by Bonferroni procedure. Haplotype constructions were analyzed using online computer platform SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [17].

Results

Characteristics of Study Subjects

General characteristics of gastric cancer cases and healthy controls were listed in Table 1. The case group comprised 98 females and 260 males aged from 29 to 82 years (mean ± SD, 56.51±10.51). The control group comprised 100 females and 312 males aged from 36 to 87 years (mean ± SD, 55.43±9.28). There were no significant differences in mean age or gender distribution between cases and controls, which suggest adequate matching based on these two variables. But there were significant differences in the distributions of smoking status, alcohol consumption, and H. pylori-positive rate between the GC patients and controls.

Table 1 Distribution of demographic variables of the gastric cancer patients and controls

Variables	Cases (n=358)	Controls (n=412)	P-Value
Gender, n (%)			
Male	260(72.6)	312(75.7)	0.326[a]
Female	98(27.4)	100(24.3)	
Age ,(years)			
Mean age(±SD)	56.51(±10.51)	55.43(±9.28)	0.131[b]
Range	29–82	36–87	
Smoking status, n (%)			
Never smoker	42(11.7)	76(18.4)	0.005 *
Ex-smoker	55(15.4)	61(14.8)	
Current smoker	235(65.6)	227(55.1)	
Missing	26(7.3)	48(11.7)	
Alcohol use, n (%)			
Yes	220(61.5)	167(40.5)	2.1×10^{-6} [a]
No	118(33.0)	187(45.4)	
Missing	20(5.6)	58(14.1)	
H. pylori, n (%)			
Positive	215(60.1)	175(42.5)	4.8×10^{-6} [a]
Negative	120(33.5)	197(47.8)	
Missing	23(6.4)	40(9.7)	

[a] χ^2 test; [b] t-test.*smoker(Ex-smoker + Current smoker) versus Never smoker

Association Between Romo1 Polymorphisms and Risk of GC

Genotype for two loci of Romo1 gene were successfully detected by PCR-DHPLC and further confirmed by subsequent sequencing (Supplemental Fig. 1–3, the genotypes of partially samples was determined by using direct sequence). The genotype distributions of two loci in the controls were in Hardy–Weinberg equilibrium (rs6060567 HWP=0.012, as shown in Table 2.). The observed allelic frequencies of the genotyped SNPs in controls showed similar results with the International

HapMap Project data for CHB. When the TT genotype was served as the reference group, the frequency of the rs660566 TC genotype was markedly higher in gastric cancer patients (36.9 %) than in control subjects (24.5 %). The TC genotype was associated with a modestly increased risk (Adjusted OR =1.525, 95 % CI=1.126–2.138, P=0.008). On the contrary, we did not find any significant difference in CC genotype frequency between gastric cancer patients and controls. We also found a strong association between the gastric cases and controls under dominant genetic model (TC+CC

Table 2 Genotype/allele distributions of Romo1 polymorphisms between GC cases and controls

Genotypes/alleles	Case, n (%)	Control, n (%)	χ2	P value	Crude OR (95%CI)	*P* value	Adjusted OR (95 % CI)
rs6060566							
T/T	221(61.7)	308(74.8)			1.000		1.000
T/C	132 (36.9)	101(24.5)	14.40	**1.48×10^{-4}**	1.821(1.334–2.487)	**0.008***	1.525(1.126–2.138)
C/C	5(1.4)	3(0.7)	1.389	0.291	2.323(0.549–9.821)	NE	
T/C+C/C	137(38.3)	104(25.2)	15.11	**1.01×10^{-4}**	1.836(1.349–2.498)	**0.013***	1.629(1.205–2.013)
T	574(80.2)	717 (87.0)			1.000		
C	142(19.8)	107(13.0)	13.25	**2.73×10^{-4}**	1.658(1.261–2.180)	NC	
rs6060567							
G/G	197(55.0)	288(69.9)			1.000		1.000
G/C	132(36.9)	104(25.2)	15.01	**1.07×10^{-4}**	1.856(1.355–2.541)	**0.001***	1.641(1.238–2.291)
C/C	29(8.1)	20(4.9)	6.28	**0.012**	2.120(1.166–3.854)	**0.023***	1.594(1.102–2.973)
G/C+CC	161(45.0)	124(30.1)	18.20	**2.01×10^{-5}**	1.898(1.412–2.552)	**0.019***	1.637(1.095–2.841)
G	526(73.5)	680(82.5)			1.000		
C	190(26.5)	144(17.5)	18.52	**1.68×10^{-5}**	1.706(1.336–2.179)	NC	

OR odds ratio, *CI* confidence interval, *NC* not calculated, *NE* not estimated

**P* value, to maintain significance after correction for multiple tests by stricter traditional Bonferroni procedure

Allelic P values were calculated by Pearson χ^2 test. Genotypic p values were estimated by unconditional logistic regression analyses, Genotypic ORs were adjusted for age, gender

Statistically significant results ($p<0.05$) are highlighted in bold

versus TT $P=0.013$, adjusted OR=1.629, 95%CI=1.205–2.013). In addition, C allele was likely to be a more susceptible risk of gastric cancer compared with T allele ($P=2.73\times10^{-4}$, OR=1.658, 95 % CI=1.261–2.180).

Moreover, another SNP rs6060567 downstream 24 bp from rs6060566, when the GG genotype were used as the reference group, significantly increased risk for gastric cancer were associated with GC genotype (Adjusted OR=1.641, 95%CI =1.238–2.291), CC genotype (Adjusted OR=1.594, 95%CI =1.102–2.973) and C carrier (Adjusted OR=1.637, 95%CI =1.095–2.841). All of which maintained significance after correction for multiple tests by stricter traditional Bonferroni procedure. However, we did not find any correlation of both rs6060566 and rs6060567 polymorphisms with histological grade of gastric cancer (Data not shown).

We further examined the association between two SNPs and gastric cancer stratified by difference gender and other environmental factors in all individuals (Table 3). The rs6060566 TC genotype and C carrier were significantly associated with higher risk of gastric cancer in smokers ,drinkers, H. Pylori- positive patients, and male cases(Adjusted OR=2.135, 95%CI =1.278–3.061, $p=0.004$; Adjusted OR=1.645, 95%CI =1.093–2.461; Adjusted OR=2.135, 95%CI =1.426–3.018; Adjusted OR=2.171, 95%CI =1.437–3.035; respectively), but no association was found in non-smokers, non-alcohol consumption, H. pylori-negative, and female patients subgroups. Similar phenomenon were observed in the rs6060567 locus. Moreover, data about dietary habit were not sufficiently standardized across studies to allow meaningful investigation of lifestyle-related modification effectively, so these parameters were not analyzed in this study.

Haplotype Analysis

The main haplotype distributions of rs6060566 and rs6060567 polymorphisms in Romo1-intron2 were summarized in Table 4. In this study, we inferred three different haplotypes. They were almost complete linkage (D′=0.989, $r^2=0.670$) (Supplemental Fig 4). The most common haplotype TG, which is formed by the common alleles of the two SNPs, displayed the strongest evidence of association with gastric cancer (uncorrected $P=1.55\times10^{-5}$), and was associated with protection against gastric cancer (OR=0.584, 95 % CI=0.457–0.746). However, another common haplotype CC, which is formed by the minor alleles of the two SNPs, appeared to be a risk haplotype for gastric cancer (uncorrected $P=9.42\times10^{-5}$, OR=1.732, 95 % CI=1.312–2.286). We also correct all P values by using the Bonferroni correction, the haplotypes TG and CC remained significant association after procedure were used. But haplotype TC did not reach the significance threshold.

Discussion

The results of this study indicated rs6060566 and rs6060567 in intron2 of Romo1 gene were associated with GC risk. As we known, it is the first report that Romo1 gene is associated

Table 3 Stratification analysis of association between the romo1 gene polymorphisms and the gastric cancer riskNo

Variables	rs6060566	No. [a]	P value[#]	AOR(95 % CI)*	rs6060567	No. [a]	P value[#]	AOR(95 % CI)*
Gender								
Male	T/T	157/237		1.000	G/G	138/228		1.000
	T/C	100/74	**0.002**	2.171(1.437–3.065)	G/C	102/72	**5.1×10^{-4}**	2.428(1.701–3.082)
	C/C	3/1	0.215	4.603(0.558–41.125)	C/C	20/12	**0.007**	2.843(1.405–4.962)
	T/C+C/C	103/75	**0.001**	2.134(1.328–2.985)	G/C+CC	122/84	**2.8×10^{-4}**	2.674(1.502–2.985)
Female	T/T	64/71		1.000	G/G	59/60		1.000
	T/C	32/27	0.523	1.432(0.673–2.522)	G/C	30/32	0.875	1.012(0.616–1.951)
	C/C	2/2	0.854	1.217(0.226–9.031)	C/C	9/8	0.814	1.203(0.582–2.988)
	T/C+C/C	34/29	0.412	1.328(0.735–2.443)	G/C+CC	39/40	0.706	1.014(0.672–2.058)
Smoking								
Smoker	T/T	215/243		1.000	G/G	177/205		1.000
	T/C	71/44	**0.004**	2.135(1.278–3.061)	G/C	101/73	**0.014**	1.712(1.136–2.412)
	C/C	4/1	0.087	4.701(0.653–42.185)	C/C	12/10	0.527	1.425(0.674–3.109)
	T/C+C/C	75/45	**0.002**	2.043(1.254–2.958)	G/C+C	113/83	**0.008**	1.622(1.182–2.096)
Non-smoker	T/T	6/18		1.000	G/G	19/47		1.000
	T/C	36/56	0.302	1.837 (0.744–4.962)	G/C	15/22	0.215	1.635(0.796–3.831)
	C/C	0/2		NE	C/C	8/7	0.125	2.905(0.692–7.963)
	T/C+C/C	36/58	0.313	1.742(0.658–5.214)	G/C+CC	23/29	0.171	1.784(0.805–4.325)
Alcohol								
Positive negative	T/T	123/112		1.000	G/G	117/108		1.000
	T/C	95/55	**0.021**	1.645(1.093–2.461)	G/C	91/50	**0.016**	1.704(1.173–2.691)
	C/C	2/0		NE	C/C	12/9	0.725	1.133(0.506–2.941)
	T/C+C/C	97/55	**0.027**	1.701(1.142–2.385)	G/C+CC	103/59	**0.022**	1.652(1.175–2.698)
	T/T	85/140		1.000	G/G	76/124		1.000
	T/C	30/45	0.821	1.103(0.762–1.753)	G/C	27/53	0.641	0.977(0.526–1.391)
	C/C	3/2	0.401	2.462(0.413–14.931)	C/C	15/10	0.069	2.327(1.105–5.658)
	T/C+C/C	33/47	0.642	1.216(0.752–2.013)	G/C+CC	42/63	0.701	1.158(0.752–1.853)
H. pylori								
Positive	T/T	113/122		1.000	G/G	110/112		1.000
	T/C	97/50	**0.001**	2.135(1.426–3.018)	G/C	92/52	**0.008**	1.925(1.259–2.874)
	C/C	5/3	0.501	1.833(0.526–7.631)	C/C	13/11	0.857	1.233(0.674–2.781)
	T/C+C/C	102/53	**0.001**	2.194(1.381–3.025)	G/C+CC	105/63	**0.012**	1.748(1.156–2.648)
Negative	T/T	88/150		1.000	G/G	76/139		1.000
	T/C	32/47	0.648	1.213(0.705–1.859)	G/C	30/50	0.647	0.998(0.525–1.783)
	C/C	0/0		NE	C/C	14/8	0.048	2.987(1.358–8.831)
	T/C+C/C	32/47		NE	G/C+CC	44/58	0.213	1.425(0.752–2.106)

[a] , cases/controls

AOR adjusted OR, ORs were adjusted for age, gender, smoking status, alcohol consumption and H. pylori infection status, *NE* not estimated

,Statistically significant results ($p<0.05$) are highlighted in bold

with gastric cancer risk in Chinese population. The two intronic SNP both confer to GC susceptibility do not directly change protein structure. However, they may influence various aspects of mRNA metabolism including alterations of regulatory RNA-binding protein sites and mRNA secondary structures. In addition, we found rs6060566 showed strong linkage disequilibrium with rs6060567. Haplotype TG and CC were associated with higher susceptibility to GC. Haplotype TC only accounted for 6.8 % of all haplotypes in GC patients and 5.0 % in controls. Therefore, haplotype analysis

Table 4 Main haplotype frequencies of rs6060566/rs6060567 genetic polymorphism between GC patients and controls*

Loci	Haplotype	Case (freq)	Control (freq)	χ2	*P* value	Pc	OR [95%CI]
rs6060566/ rs6060567	CC	141.00(0.197)	101.81(0.124)	15.274	$\mathbf{9.42\times10^{-5}}$	$\mathbf{5.75\times10^{-4}}$	1.732[1.312~2.286]
	TC	49.00(0.068)	41.19 (0.050)	2.306	0.129	NS	1.390[0.907~2.131]
	TG	526.00(0.735)	677.81(0.823)	18.717	$\mathbf{1.55\times10^{-5}}$	$\mathbf{9.30\times10^{-5}}$	0.584 [0.457~0.746]

Pc, Corrected P value (after Bonferroni multiple adjustment); NS, not significant; OR, odds ratio; CI, confidence interval

P value was calculated by Fisher's exact test

* Frequency results of <0.03 in both GC and controls have been excluded

Statistically significant results ($p<0.05$) are highlighted in bold

confirms the results of genotyping and highlights a contribution of Romo1 gene to gastric cancer development.

Previous studies showed increased Romo1 expression was observed in various cancer cell lines, and enforced Romo1 expression induces ROS production in the mitochondria leading to massive cell death [10, 18]. Our study confirmed rs6060566 and rs6060567 loci in Romo1 gene were associated with GC susceptibility in a Chinese population, which indicated the genetic variants may affect the Romo1 induced production of ROS during cancer progression and increase GC cell malignancy. Over expression of Romo1 triggered premature senescence and both nuclear and mitochondrial DNA damage by ROS production, which can results in mtDNA instability and mitochondrial dysfunction [19, 20]. Over expression Romo1 can increased hepatocellular carcinoma (HCC) cells' invasive activity, and high levels of Romo1 expression correlated with vascular invasion by the tumors, reduced differentiation, and larger tumor size [21]. These finds suggested that the change of Romo1 expression level contributes toward various cancer occurrence and development. Furthermore, it is discovered Romo1 gene evolution is highly conserved, and its orthologs are 100 % identical at the amino acid level in all analyzed mammalian species [22], any minor changes in Romo1 may lead to serious consequences. In addition, some study also demonstrated drug resistance derived from increased Romo1 expression is one of the malignant phenotypes of tumors [18]. The aforementioned findings suggest that the Romo1 gene confer susceptibility to tumors are more complex than initially thought. Thus more comprehensive studies warranted exploring the gene mutation and protein expression may provide new clinical implication in prevention and diagnosis of GC.

However, the results to date suggest the gene mutation in plasma may have limited clinical utility in the detection of carcinoma. Gastric tumor with heterogeneity may confound genetic analysis. DHPLC could be used to detect these heteroplasmic species and therefore act as a rapid screening test for gene mutation [23].

Furthermore,in current study,we found that higher frequencies of individual in the cases group had consumed cigarette, alcohol use, and H. Pylori-positive rate, compared with the controls (Table 1). The results of molecular epidemiology studies demonstrated that smoking and alcohol consumption are main known etiological factors of some tumors,up-regulation of nuclear hypoxia-inducible factor (HIF)-1αcigarette is induced by tobacco consumption, nicotine-induced over-expression of HIF-1α was dependent on mitochondria-derived reactive oxygen species (ROS) [24], the levels of HIF-1 up-regulation with the progression of the pathological stage of tumors and have been positively related to tumors aggressiveness and a poor prognosis [25]. Alcohol use can increase protein expression levels of c-fos and c-jun proto-oncogenes [26, 27]. Long-term cigarettes smoking and alcohol drinking have been shown to contribute to carcinogenesis. As we all know,International Agency for Research on Cancer have been defined H. Pylori as a class I definite carcinogen,and H. Pylori may acts as the initiating agent for gastric cancer development [28]. Higher incidence rates in males than those in females may be partially explained by the differences of lifestyle between different gender (i.e. higher rates for alcohol use and cigarette consumption). These finding suggested that tobacco consumption, alcohol drinking and H. pylori IgG sero-positivity are highly associated with increased risk for gastric cancer. Combined analyzing the Romo1 SNPs together with these factors may be useful for predicting the risk of gastric cancer. Till now, there are few reports about association of mutation in mitochondria modification protein-Romo1 gene with tumors risk in Chinese population. This study may provide some genetic information and environment factors (smoking, alcohol consumption etc.) for understanding it. Of course, we should be considered some potential limitations in our study. First, relatively small sample size in our study may not have been large enough to detect variants with low frequency (rs6060566), so, further replication in different population will be required to assess whether this association is sufficiently robust. On the other hand, the same polymorphic locus under different genetic background may display different expression patterns. Thus, the effects of these gene polymorphisms on the occurrence and development of GC are still need further investigation for the clinically useful genetic marker. Second, the self-reported smoking and alcohol details were not validated in this study, and the amount smoked per

day and drinking per day were not calculated, the parameters were only classified as smoker/non-smoker, drinker/non-drinker, respectively. Finally, no detailed information on nutritional factors was available (i.e. intake of salt, anti-oxidative vitamins, and meals etc.) the SNPs that were selected in current study located in the intron region of gene, thus, we should add potential functional SNPs are warranted to identify the role of Romo1 polymorphisms and gene–environment interactions in gastric carcinogenesis in future studies.

Conclusion

These findings suggested genetic polymorphisms of Romo1 gene and their estimated haplotypes are related to predisposition of GC in Chinese population, tobacco consumption, alcohol use, and H. Pylori infection status are also highly associated with increased risk for gastric cancer development. But more studies based on larger sample size, case-control design, and stratification by ethnic and clinical outcomes are still needed in future research.

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Conflict of Interest No conflicts of interest were declared in relation to this article.

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