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ORIGINAL ARTICLE

Genetic variations associated with telomere length confer risk of gastric cardia adenocarcinoma

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Abstract

Background Aberrant telomere lengthening is a critical feature of malignant cells. Short leukocyte telomere length (LTL) confers elevated risk of gastric cardia adenocarcinoma (GCA). Multiple genome-wide association studies (GWAS) identified various single-nucleotide polymorphisms (SNPs) associated with LTL in different ethnic populations. However, it remains largely unexplored how these genetic variants are involved in GCA susceptibility.

Methods We systematically screened GWAS-identified candidate SNPs and tested the impact of 30 polymorphisms in genes associated with interindividual LTL variation on GCA using two-stage case–control comparisons consisting of 1024 GCA patients and 1118 controls.

Results We observed that *CXCR4* rs6430612, *TERT* rs10069690, and rs2853676 as well as *VPS34* rs2162440 are significantly associated with GCA development. A 0.64-fold decreased risk of GCA is associated with the *CXCR4* rs6430612 CT genotype compared with the CC genotype ($P=0.002$). On the contrary, the *TERT* rs10069690 TT genotype carriers had a 1.83-fold increased risk to develop GCA compared to the CC genotype carriers ($P=5.8\times10^{-6}$). We also detected a 2.17-fold increased OR for GCA that was associated with the *TERT* rs2853676 TT genotype ($P=2.6\times10^{-6}$). In addition, the odds of having the *VPS34* rs2162440 GA genotype in GCA patients were 1.35 compared with the GG genotype ($P=0.002$). In stratified analyses, the association between *TERT* rs10069690 polymorphism and GCA was more pronounced in nonsmokers ($P_{\text{interaction}}=9.7\times10^{-5}$) and nondrinkers ($P_{\text{interaction}}=4.6\times10^{-5}$).

Conclusions Our results highlight the importance of both LTL and LTL-related genetic variants to GCA predisposition.

Keywords Telomere · GCA · CXCR4 · TERT · VPS34 · Genetic polymorphism

Introduction

Estimated 951,600 new gastric cancer cases and 723,100 deaths occurred in 2012 worldwide [1]. Gastric cancer incidences vary widely across countries, and in general, incidence rates are highest in Eastern Asia, Central and Eastern Europe, and South America, and lowest in Northern America and most parts of Africa [1]. According to National Central Cancer Registry of China (NCCRC) updated nationwide statistics of cancer incidence and mortality in China using population-based cancer registration data in 2013, gastric cancer was the second most common cancer, accounting for about 11.6% (427,100) of all new cancers [2] and the third most common cause of cancer death (301,200) of all cancer deaths in China [2]. Gastric cancer has two main subtypes including cardia (proximal, gastroesophageal junction) and noncardia (fundus, body, distal, and lesser or greater curvature). Gastric cardia carcinoma (GCA) commonly occurs in the 1 cm proximal and 2 cm distal region of the esophagogastric junction. Using Cancer Incidence in Five Continents Volume X, it was estimated that there were 260,000 cases of GCA (age standardized incidence rates: 3.3 per 100,000) worldwide in 2012 [3]. Dissimilar to gastric cancer at other sites, GCA is a special type of gastric cancer having its own

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epidemiological characteristics, etiology, pathogenesis, and clinical manifestations [4]. Cigarette smoking, heavy alcohol consumption, dietary carcinogen exposure, low-intake of fruits and vegetables, and gastroesophageal reflux disease have been identified as GCA environmental risk factors [5–8]. Interestingly, accumulated evidences demonstrated that genetic makeups also play a part in etiology of GCA [9–12].

As nucleoprotein structures capping and protecting the ends of chromosomes, telomeres are engaged in a host of cellular functions [13, 14]. Telomeres shorten with each cell division and leucocyte telomere length has been shown to decrease with age at a rate of 20–40 base pairs (bp) per year [15, 16]. Leucocyte telomere length is a predictor for a number of common age-related diseases including cancers [17]. Consistent to these findings, we previously showed that short leucocyte telomere length contributes to increased susceptibility to GCA [10]. Telomeres length is a heritable trait, with heritability ranging from 44 to 80% and regulated by multiple genes [18, 19]. Several genome-wide association studies (GWAS) and candidate gene studies identified dozens of single-nucleotide polymorphisms (SNPs) associated with leucocyte telomere length in different ethnic populations [20–29]. Intriguingly, multiple telomere length-related SNPs confer risk of different malignancies, such as prostate cancer, ovarian cancer, leukemia, colorectal cancer, glioma, and pancreatic cancer. Nevertheless, the mechanisms by which common variants associated with leucocyte telomere length impact development of GCA are still unclear.

In view of the importance of telomeres in carcinogenesis, we hypothesized that the aforementioned telomere length-related genetic polymorphisms may contribute to GCA risk in Chinese populations. To test this hypothesis, we conducted a large case–control study of GCA with two Chinese populations from different regions of China.

Materials and methods

Study case–control sets

This study consisted of two case–control sets (Table 1): (a) Shandong set (discovery set): 584 patients with GCA from Shandong Cancer Hospital affiliated to Shandong University (Jinan, Shandong Province, China) and sex- and age-matched (±5 years) 568 controls. Patients were recruited between August 2012 and January 2018 at Shandong Cancer Hospital. Exclusion criteria were that patients with a second primary tumor or the primary tumor outside of gastric cardia. Control subjects were randomly selected from a pool of 6800 individuals from a comprehensive physical examination conducted in Jinan city and the surrounding areas during the same time period as the patients were collected. Part of the case–control set has been reported previously [10]. (b) Jiangsu set (validation set): 440 GCA patients from Huaian No. 2 Hospital (Huaian, Jiangsu Province, China) and sex- and age-matched 500 controls. Patients were consecutively recruited between June 2010 and January 2018 at Huaian No. 2 Hospital. The controls were cancer-free individuals based on a physical examination, randomly selected from a pool of 4000 subjects which were recruited from the same hospital during the same time period as the patients were

Table 1 Distribution of selected characteristics among GCA cases and controls

Variables	Shandong set (discovery set)			Jiangsu set (validation set)		
	Cases	Controls	P^a	Cases	Controls	P^a
	No. (%)	No. (%)		No. (%)	No. (%)	
	$n=584$	$n=568$		$n=440$	$n=500$	
Age (year)			0.458			0.320
≤ 66	314 (53.8)	293 (51.6)		226 (51.4)	265 (48.2)	
> 66	270 (46.2)	275 (48.4)		214 (48.6)	285 (51.8)	
Sex			0.598			0.512
Male	494 (84.6)	474 (83.5)		367 (83.4)	450 (81.8)	
Female	90 (15.4)	94 (16.5)		73 (16.6)	100 (18.2)	
Smoking status			< 0.001			< 0.001
No	309 (52.9)	449 (79.0)		213 (48.4)	412 (74.9)	
Yes	275 (47.1)	119 (21.0)		227 (51.6)	138 (25.1)	
Drinking status			0.006			0.005
No	349 (59.8)	384 (97.6)		238 (54.1)	346 (62.9)	
Yes	235 (40.2)	184 (32.4)		202 (45.9)	204 (37.1)	

GCA gastric cardia adenocarcinoma

[a]Two-sided Chi-square test

collected. The diagnosis of all patients was histologically confirmed. Individuals who smoked one cigarette per day for over 1 year were considered as smokers. Subjects were considered as alcohol drinkers, if they drank at least once per week. All subjects were ethnic Han Chinese. At recruitment, informed consent was obtained from each subject and each participant was then interviewed to collect detailed information on demographic characteristics, such as sex, age, cigarette smoking and alcohol drinking. This study was approved by the institutional Review Boards.

Genetic polymorphism selection and genotyping

After literature searches with search terms of "telomere length", "polymorphism", "variant", "SNP", as well as their combinations, we identified 88 SNPs significantly associated with telomere length published before April 2018. By systematically screening CHB (Chinese Han Beijing) and CHS (Han Chinese South) data of the 1000 genomes project, we excluded 53 SNPs which either have minor allele frequencies (MAF) < 0.03 in Chinese populations or show strong linkage disequilibrium with other candidate SNP(s) with an r^2 threshold of 0.80. Five polymorphisms were excluded, since they are unable to be genotyped using the iPLEX Sequenom MassARRAY platform (Sequenom Inc., San Diego, CA, USA). Finally, a total of 30 candidate genetic variants were included in the study (Table 1).

Thirty telomere length-related SNPs were first analyzed in the Shandong case–control set (discovery set). *CXCR4* rs6430612, *TERT* rs10069690 and rs2853676, and *VPS34* rs2162440 were then examined in the Jiangsu case–control validation set. Genotypes of all these polymorphisms were examined using the MassARRAY platform as previously reported [30–32]. A 15% random sample was reciprocally tested and the reproducibility was 100%.

Statistics

Pearson's chi-square test was used to examine the differences in demographic variables, smoking status, drinking status and genotype distributions of all 30 SNPs between GCA cases and controls. The associations between telomere length-related SNPs and GCA risk were estimated by odds ratios (ORs) and their 95% confidence intervals (95% CIs) computed by logistic regression models. All ORs were adjusted for age, sex, smoking or drinking status, where it was appropriate. The association of SNPs with telomere length was assessed using linear regression adjusted for age, sex, smoking and drinking status. During meta-analyses, a fixed effect model (the Mantel–Haenszel method) was performed to calculate the combined OR using Stata Statistical package (version 11.0; Stata Corp.). Bonferroni correction was used for multiple comparisons. All statistical tests were two-sided and were performed using SPSS 16.0 (SPSS Inc.).

Results

Subject characteristics

As shown in Table 1, age and sex distributions were adequately matched in either Shandong set or Jiangsu set. There were no significant statistical differences in the distributions of median age and sex between GCA patients and controls in both case–control sets (all $P > 0.05$). However, smokers and drinkers accounted for a higher proportion among GCA patients compared with those in controls (Shandong set: smokers: 47.1% vs. 21.0%, $P < 0.001$; drinkers: 40.2% vs. 32.4%, $P = 0.006$; Jiangsu set: smokers: 51.67% vs. 25.1%, $P < 0.001$; drinkers: 45.9% vs. 37.1%, $P = 0.005$) (Table 1).

Allelic frequencies of 30 telomere length-related SNPs in the discovery set

Allele frequencies of 30 telomere length-related genetic polymorphisms in cases and controls are showed in Table 2. All observed genotype frequencies in both controls and patients conform to Hardy–Weinberg equilibrium. Distributions of the genotypes were then compared among cases and controls. Frequencies of *CXCR4* rs6430612 C and T alleles among patients differed significantly from those among controls ($\chi^2 = 7.74$, $P = 0.005$), with the frequency of T allele being significantly lower among patients than among controls (4.6% vs. 7.4%). On the contrary, allele frequencies of the *TERT* rs10069690 and rs2853676 as well as *VPS34* rs2162440 genetic variants were significantly different among cases and controls (rs10069690: $\chi^2 = 7.11$, $P = 0.008$; rs2853676: $\chi^2 = 18.65$, $P = 1.6 \times 10^{-5}$; rs2162440: $\chi^2 = 4.21$, $P = 0.040$), with the frequency of the rs10069690 T, rs2853676 T as well as rs2162440 A allele being higher in cases than in controls (rs10069690: 21.2% vs. 16.7%; rs2853676: 19.8% vs. 12.9%; rs2162440: 22.6% vs. 19.1%). However, no statistically significant allele differences of other SNPs were observed between GCA cases and controls (all $P > 0.05$) (Table 2).

Association between telomere length-related polymorphisms and GCA risk in both discovery and validation case–control sets

Unconditional logistic regression analysis was used to estimate associations between genotypes of telomere length-related *CXCR4* rs6430612, *TERT* rs10069690 and rs2853676, and *VPS34* rs2162440 polymorphisms and GCA risk in Shandong set (Table 3). The *CXCR4* rs6430612 T

Table 2 Association between GCA susceptibility and telomere length-related genetic variations from previously published studies (Shandong discovery set)

Genes	SNP IDs	Location[a]	Alleles[b]	MAF of cases/controls	OR[c]	95% CI[c]	*P*[c]	Effect allele[d] and reference
ACYP2	rs11125529	Chromosome 2:54475866	C/A	0.116/0.123	0.94	0.74–1.21	0.631	A; Ojha et al. [20]
DDX18	rs6712766	Chromosome 2:118611789	G/A	0.049/0.053	0.91	0.63–1.32	0.628	A; Julin et al. [21]
CXCR4	rs6430612	Chromosome 2:137006198	C/T	0.046/0.074	0.62	0.43–0.87	0.006	T; Levy et al. [22]
TERC	rs12696304	Chromosome 3:169481271	G/C	0.299/0.289	1.05	0.88–1.25	0.619	G; Shen et al. [23]
TERC	rs10936599	Chromosome 3:169492101	T/C	0.448/0.453	0.98	0.83–1.16	0.829	T; Walsh et al. [29]
TERC	rs16847897	Chromosome 3:169568116	G/C	0.379/0.394	0.94	0.80–1.11	0.463	G; Codd et al. [26]
TERC	rs1920116	Chromosome 3:169579971	G/A	0.424/0.411	1.05	0.89–1.24	0.532	A; Jones et al. [24]
TERT	rs4246742	Chromosome 5:1267356	T/A	0.318/0.333	0.93	0.79–1.11	0.422	T; Terry et al. [25]
TERT	rs13172201	Chromosome 5:1271661	C/T	0.146/0.145	1.01	0.79–1.27	0.969	T; Bao et al. [36]
TERT	rs10069690	Chromosome 5:1279790	C/T	0.212/0.167	1.35	1.10–1.67	0.005	C; Julin et al. [21]
TERT	rs2736100	Chromosome 5:1286516	C/A	0.418/0.412	1.01	0.86–1.20	0.872	C; Ojha et al. [20]
TERT	rs2853677	Chromosome 5:1287194	A/G	0.355/0.362	0.97	0.82–1.15	0.728	G; Bao et al. [36]
TERT	rs2853676	Chromosome 5:1288547	C/T	0.198/0.129	1.65	1.32–2.08	1.6×10^{-5}	C; Julin et al. [21]
TERT	rs2736098	Chromosome 5:1294086	C/T	0.344/0.343	0.99	0.83–1.19	0.937	T; Julin et al. [21]
TERT	rs451360	Chromosome 5:1319680	C/A	0.095/0.089	1.08	0.81–1.43	0.622	C; Bao et al. [36]
TERT	rs402710	Chromosome 5:1320722	C/T	0.319/0.316	1.03	0.86–1.23	0.774	C; Bao et al. [36]
CLPTM1L	rs401681	Chromosome 5:1322087	C/T	0.339/0.333	1.03	0.87–1.24	0.711	T; Julin et al. [21]
SLC44A4	rs2736428	Chromosome 6:31843924	C/T	0.333/0.324	1.03	0.87–1.23	0.705	C; Levy et al. [22]
LOC105376013	rs10511887	Chromosome 9:31837334	A/G	0.268/0.255	1.06	0.88–1.28	0.536	A; Codd et al. [26]
OBFC1	rs10883943	Chromosome 10:105651416	G/T	0.327/0.303	1.14	0.94–1.36	0.177	G; Julin et al. [21]
OBFC1	rs4387287	Chromosome 10:105677897	A/C	0.137/0.154	0.88	0.70–1.10	0.263	A; Levy et al. [22]
GRIA4	rs610160	Chromosome 11:105696895	T/C	0.162/0.167	0.97	0.77–1.21	0.780	C; Codd et al. [26]
CSNK2A2	rs74019828	Chromosome 16:58209274	G/A	0.089/0.111	0.87	0.66–1.14	0.301	A; Julin et al. [21]
MPHOSPH6	rs2967374	Chromosome 16:82209861	G/A	0.235/0.268	0.83	0.68–1.01	0.065	A; Julin et al. [21]
CTC1	rs3027234	Chromosome 17:8136092	C/T	0.041/0.042	0.98	0.65–1.48	0.918	C; Julin et al. [21]
MEOX1	rs8081000	Chromosome 17:41715233	A/G	0.414/0.390	1.10	0.93–1.29	0.282	A; Levy et al. [22]

Table 2 (continued)

Genes	SNP IDs	Location[a]	Alleles[b]	MAF of cases/controls	OR[c]	95% CI[c]	*P*[c]	Effect allele[d] and reference
VPS34	rs2162440	Chromosome 18:35214006	G/A	0.226/0.191	1.23	1.01–1.51	0.046	G; Mangino et al. [27]
CCBE1	rs1791285	Chromosome 18:57366183	T/C	0.080/0.081	0.99	0.74–1.34	0.986	C; Julin et al. [21]
ADA	rs73598374	Chromosome 20:43280227	C/T	0.023/0.019	1.23	0.71–2.15	0.466	C; Concetti et al. [28]
RTEL1	rs6010620	Chromosome 20:62309839	A/G	0.267/0.236	1.18	0.98–1.43	0.088	A; Walsh et al. [29]

GCA gastric cardia adenocarcinoma, *SNP* single-nucleotide polymorphism, *MAF* minor allele frequency, *OR* odds ratio, *CI* confidence interval

[a]Reference genome GRCh37.p13

[b]Major allele/minor allele for each polymorphism

[c]Data were calculated by logistic regression with adjustment for age, sex, smoking and drinking status

[d]The effect allele possibly associated with increased leukocyte telomere length

allele was showed to be protective allele; subjects having the CT genotype had an OR of 0.66 (95% CI 0.45–0.96; $P=0.029$) for developing GCA compared with subjects having the CC genotype. It was observed that a significantly increased risk of developing GCA was associated with the *TERT* rs10069690 TT genotype (OR 1.69; 95% CI 1.20–2.37; $P=0.003$) compared with the rs10069690 CC genotype. However, the *TERT* rs10069690 CT heterozygous genotype showed no effect on GCA risk. In addition, individuals with the *TERT* rs2853676 CT or TT genotype also showed significantly increased GCA risk compared with those with the rs2853676 CC genotype (OR 1.50; 95% CI 1.15–1.95; $P=0.003$; OR 2.20; 95% CI 1.41–3.45; $P=5.8\times10^{-4}$) (Table 3). A significantly increased OR was associated with the *VPS34* rs2162440 GA genotype (OR 1.31, 95% CI 1.02–1.68, $P=0.037$), but was not associated with the rs2162440 AA genotype ($P=0.348$). All ORs were adjusted for sex, age, smoking and alcohol drinking status. We also analyzed the association of 30 SNPs with telomere length in cases and controls of Shandong set. As shown in Supplementary Table 1, we found that *CXCR4* rs6430612, *TERT* rs10069690, and rs2853676 as well as *VPS34* rs2162440 polymorphisms are significantly associated with telomere length. In detail, the *CXCR4* rs6430612 T allele was associated with long telomere length and showed to be protective allele. On the contrary, the minor alleles of *TERT* rs10069690 and rs2853676 as well as *VPS34* rs2162440 are all associated with short telomere length. However, we did not find significant correlation between polymorphisms and telomere length in controls. Similar association trends between SNPs and telomere length in controls exist as it does among GCA cases (Supplementary Table 2).

In the Jiangsu validation set, the significant associations between *CXCR4* rs6430612, *TERT* rs10069690 and rs2853676, and *VPS34* rs2162440 polymorphisms and GCA risk were also observed (Table 3). Logistic regression analyses revealed that individuals with *CXCR4* rs6430612 CT genotype were significantly associated with decreased GCA risk (OR 0.60, 95% CI 0.38–0.94, $P=0.025$). Carriers of the *TERT* rs10069690 TT genotype showed significantly elevated risk to develop GCA compared with the rs10069690 CC carriers (OR 2.02, 95% CI 1.34–3.04, $P=8.0\times10^{-4}$). Similarly, significantly increased GCA risk was observed among *TERT* rs2853676 CT or TT carriers compared to individuals with the s2853676 CC genotype (OR 1.76, 95% CI 1.31–2.35, $P=1.6\times10^{-4}$; OR 2.12, 95% CI 1.32–3.38, $P=0.002$). Moreover, the *VPS34* rs2162440 GA genotype was also associated with 1.40-fold (95% CI 1.07–1.84; $P=0.015$) elevated GCA risk. However, this association was not statistically significant for the rs2162440 AA genotype ($P=0.211$).

In the pooled analyses, we found that reduced GCA risk was associated with the *CXCR4* rs6430612 CT genotype (OR 0.64, 95% CI 0.78–0.85, $P=0.002$) (Table 3). The *TERT* rs10069690 CT or TT genotype carriers had a 1.24-fold or 1.83-fold increased risk to develop GCA compared to the rs10069690 CC genotype carriers (95% CI 1.03–1.50, $P=0.023$ or 95% CI 1.41–2.38, $P=5.8\times10^{-6}$). The *TERT* rs2853676 CT or TT genotype was also significantly associated with elevated GCA risk (OR 1.62, 95% CI 1.33–1.97, $P=1.3\times10^{-6}$; OR 2.17, 95% CI 1.57–3.00, $P=2.6\times10^{-6}$) compared to the rs2853676 CC genotype. In addition, the odds of having the *VPS34* rs2162440 GA genotype in GCA patients was 1.35 (95% CI 1.12–1.62, $P=0.002$) compared with the rs2162440 GG genotype. As shown in Supplementary Fig. 1, meta-analyses demonstrated that all four SNPs also significantly contributed to GCA risk (*CXCR4* rs6430612: OR 0.62, 95% CI 0.47–0.83; *TERT* rs10069690: OR 1.36, 95% CI 1.14–1.63; *TERT* rs2853676: OR 1.75,

Table 3 Genotype frequencies of *CXCR4, TERT* and *VPS34* SNPs among cases and controls and their association with GCA risk

Genes	SNP IDs	Shandong set				Jiangsu set			
		Cases, no. (%)	Controls, no. (%)	OR[1] (95% CI)	*P*	Cases, no. (%)	Controls, no. (%)	OR[1] (95% CI)	*P*
CXCR4	rs6430612	*n* = 582	*n* = 568			*n* = 438	*n* = 550		
	CC	530 (91.0)	490 (86.3)	1.00 (reference)		406 (92.7)	482 (87.6)	1.00 (reference)	
	CT	51 (8.8)	72 (12.7)	0.66 (0.45–0.96)	0.029	32 (7.3)	63 (11.5)	0.60 (0.38–0.94)	0.025
	TT	1 (0.2)	6 (1.0)	NC	NC	0 (0.0)	5 (0.9)	NC	NC
	C allele			1.00 (reference)				1.00 (reference)	
	T allele			0.62 (0.43–0.87)	0.006			0.58 (0.39–0.86)	0.005
TERT	rs10069690	*n* = 584	*n* = 568			*n* = 440	*n* = 550		
	CC	367 (62.8)	390 (68.7)	1.00 (reference)		280 (63.7)	394 (71.6)	1.00 (reference)	
	CT	185 (31.7)	166 (29.2)	1.19 (0.92–1.53)	0.189	137 (31.1)	148 (26.9)	1.30 (0.98–1.71)	0.067
	TT	32 (5.5)	12 (2.1)	1.69 (1.20–2.37)	0.003	23 (5.2)	8 (1.5)	2.02 (1.34–3.04)	8.0×10^{-4}
	C allele			1.00 (reference)				1.00 (reference)	
	T allele			1.35 (1.10–1.67)	0.005			1.69 (1.36–2.04)	7.4×10^{-4}
TERT	rs2853676	*n* = 584	*n* = 568			*n* = 440	*n* = 550		
	CC	379 (64.9)	427 (75.1)	1.00 (reference)		289 (65.7)	431 (78.4)	1.00 (reference)	
	CT	179 (30.7)	135 (23.8)	1.50 (1.15–1.95)	0.003	133 (30.2)	113 (20.5)	1.76 (1.31–2.35)	1.6×10^{-4}
	TT	26 (4.5)	6 (1.1)	2.20 (1.41–3.45)	5.8×10^{-4}	18 (4.1)	6 (1.1)	2.12 (1.32–3.38)	0.002
	C allele			1.00 (reference)				1.00 (reference)	
	T allele			1.65 (1.32–2.08)	1.6×10^{-5}			1.76 (1.45–2.13)	4.9×10^{-6}
VPS34	rs2162440	*n* = 575	*n* = 560			*n* = 440	*n* = 550		
	GG	343 (59.6)	369 (65.9)	1.00 (reference)		256 (58.2)	368 (66.9)	1.00 (reference)	
	GA	204 (35.5)	168 (30.0)	1.31 (1.02–1.68)	0.037	157 (35.7)	162 (29.5)	1.40 (1.07–1.84)	0.015
	AA	28 (4.9)	23 (4.1)	1.15 (0.86–1.53)	0.348	27 (6.1)	20 (3.6)	1.22 (0.89–1.68)	0.211
	G allele			1.00 (reference)				1.00 (reference)	
	A allele			1.23 (1.01–1.51)	0.046			1.47 (1.20–1.79)	0.006

Table 3 (continued)

Genes	SNP IDs	Combined samples			
		Cases, no. (%)	Controls, no. (%)	OR[1] (95% CI)	*P*
CXCR4	rs6430612	*n* = 1020	*n* = 1118		
	CC	936 (91.8)	972 (86.9)	1.00 (reference)	
	CT	83 (8.1)	135 (12.1)	0.64 (0.78–0.85)	0.002
	TT	1 (0.1)	11 (1.0)	NC	NC
	C allele			1.00 (reference)	
	T allele			0.61 (0.51–0.78)	1.4×10^{-4}
TERT	rs10069690	*n* = 1024	*n* = 1118		
	CC	647 (63.2)	784 (70.1)	1.00 (reference)	
	CT	322 (31.4)	314 (28.1)	1.24 (1.03–1.50)	0.023
	TT	55 (5.4)	20 (1.8)	1.83 (1.41–2.38)	5.8×10^{-6}
	C allele			1.00 (reference)	
	T allele			1.53 (1.32–1.85)	4.8×10^{-6}
TERT	rs2853676	*n* = 1024	*n* = 1118		
	CC	668 (65.2)	858 (76.7)	1.00 (reference)	
	CT	312 (30.5)	248 (22.2)	1.62 (1.33–1.97)	1.3×10^{-6}
	TT	44 (4.3)	12 (1.1)	2.17 (1.57-3.00)	2.6×10^{-6}
	C allele			1.00 (reference)	
	T allele			1.82 (1.53–2.01)	5.2×10^{-7}
VPS34	rs2162440	*n* = 1009	*n* = 1110		
	GG	599 (59.4)	737 (66.4)	1.00 (reference)	
	GA	361 (35.8)	330 (29.7)	1.35 (1.12–1.62)	0.002
	AA	49 (4.8)	43 (3.9)	1.18 (0.96–1.46)	0.125
	G allele			1.00 (reference)	
	A allele			1.07 (1.19–1.55)	0.004

SNP single-nucleotide polymorphism, *GCA* gastric cardia adenocarcinoma, *NC* not calculated, *OR* odds ratio, *CI* confidence interval

[1]Data were calculated by logistic regression with adjustment for age, sex, smoking and drinking status

Table 4 Risk of GCA associated with *CXCR4, TERT*, and *VPS34* SNPs by age, sex, smoking, and drinking status

Variables	*CXCR4* rs6430612					*TERT* rs10069690				
	CC[a]	CT + TT[a]	OR[b] (95% CI)	*P*	$P_{interaction}$	CC[a]	CT + TT[a]	OR[b] (95% CI)	*P*	$P_{interaction}$
Age (year)					0.058					0.080
≤ 66	498/477	40/81	0.44 (0.29–0.68)	1.5×10^{-4}		341/381	199/177	1.14 (0.87–1.48)	0.349	
> 66	438/495	44/65	0.84 (0.55–1.28)	0.413		306/403	178/157	1.50 (1.14–1.96)	0.004	
Sex					0.025					0.771
Male	793/800	64/124	0.51 (0.37–0.72)	1.2×10^{-4}		549/648	312/276	1.29 (1.05–1.60)	0.017	
Female	143/172	20/22	0.86 (0.44–1.71)	0.674		98/136	65/58	2.36 (1.41–3.95)	0.001	
Smoking status					0.928					9.7×10^{-5}
Nonsmoker	475/745	47/116	0.58 (0.40–0.83)	0.003		319/632	203/229	1.78 (1.40–2.26)	3.3×10^{-6}	
Smoker	461/227	37/30	0.53 (0.31–0.90)	0.018		328/152	174/105	0.82 (0.59–1.14)	0.232	
Alcohol drinking					0.937					4.6×10^{-5}
No	534/627	53/103	0.60 (0.42–0.85)	0.005		366/538	221/192	1.75 (1.37–2.22)	5.6×10^{-6}	
Yes	402/345	31/43	0.51 (0.29–0.90)	0.020		281/246	156/142	0.78 (0.55–1.10)	0.150	
Variables	*TERT* rs2853676					*VPS34* rs2162440				
	CC[a]	CT + TT[a]	OR[b] (95% CI)	*P*	$P_{interaction}$	GG[a]	GA + AA[a]	OR[b] (95% CI)	*P*	$P_{interaction}$
Age (year)					0.156					0.204
≤ 66	361/445	179/113	2.01 (1.51–2.69)	2.3×10^{-6}		307/372	222/181	1.39 (1.07–1.80)	0.015	
> 66	307/413	177/147	1.68 (1.28–2.22)	2.1×10^{-4}		292/365	188/192	1.24 (0.95–1.61)	0.114	
Sex					0.335					0.861
Male	556/698	305/226	1.80 (1.44–2.24)	1.9×10^{-7}		503/608	343/309	1.31 (1.06–1.61)	0.011	
Female	112/160	51/34	2.27 (1.30–3.95)	0.004		96/129	67/64	1.45 (0.90–2.34)	0.123	
Smoking status					0.863					0.332
Nonsmoker	333/660	189/201	1.90 (1.48–2.44)	4.5×10^{-7}		305/575	210/280	1.41 (1.12–1.79)	0.004	
Smoker	335/198	167/59	1.58 (1.10–2.28)	0.014		294/162	200/93	1.24 (0.89–1.73)	0.202	
Alcohol drinking					0.881					0.126
No	382/555	205/175	1.85 (1.44–2.36)	1.1×10^{-6}		334/487	246/238	1.48 (1.18–1.87)	0.001	
Yes	286/303	151/85	1.65 (1.13–2.40)	0.010		265/250	164/135	1.05 (0.74–1.49)	0.772	

[a]Number of case patients with the genotype/number of control subjects with the genotype(s)

[b]Data were calculated by logistic regression, adjusted for sex, age, smoking and drinking status, where it was appropriate

95% CI 1.45–2.11; *VPS34* rs2162440: OR 1.39, 95% CI 1.16–1.65).

Stratified analyses of associations between *CXCR4*, *TERT* and *VPS34* polymorphisms and GCA risk

The risk of GCA associated with the *CXCR4, TERT* and *VPS34* polymorphisms was further examined by stratifying for age, sex, smoking and alcohol drinking status using the combined data of two case–control sets (Table 4). For the *CXCR4* SNP, a significantly reduced risk of GCA associated with the rs6430612 CT or TT genotype compared with the CC genotype was observed for the group aged 66 years or younger (OR 0.44, 95% CI 0.29–0.68, $P = 1.5\times10^{-4}$), but not for the group aged older than 66 years ($P = 0.413$). Compared with the CC genotype, a decreased risk of GCA was only associated with *CXCR4* CT or TT genotype for the male group (OR 0.51, 95% CI 0.37–0.72, $P = 1.2\times10^{-4}$), but not among female subjects ($P = 0.674$). There was statistically significant gene–age interaction for the *CXCR4* SNP ($P_{interaction} = 0.025$). In stratified analyses with smoking or alcohol drinking status, the *CXCR4* rs6430612 polymorphism was significantly associated with decreased risk in smokers, nonsmokers, drinkers, or nondrinkers (all $P < 0.05$) (Table 4).

For the *TERT* rs10069690 genetic variant, carriers of the rs10069690 CT or TT genotype and aged older than 66 years showed significantly elevated risk to develop GCA compared with the rs10069690 CC carriers (OR 1.50, 95% CI 1.14–1.96, $P = 0.004$). However, no such association was found for the group aged 66 years or younger ($P = 0.349$). It was observed that an increased risk of GCA was associated with the rs10069690 genotype in both males and females (both $P < 0.05$). Stratified analyses with smoking or drinking status showed that significantly elevated ORs for GCA development

were found in nonsmokers (OR 1.78, 95% CI 1.40–2.26, $P = 3.3 \times 10^{-6}$) as well as nondrinkers (OR 1.75, 95% CI 1.37–2.22, $P = 5.6 \times 10^{-6}$). However, there was no significantly increased risk for smokers or drinkers with the rs10069690 CT or TT genotype compared with smokers or drinkers with the CC genotype ($P = 0.232$, $P = 0.150$). A statistically significant gene–smoking or gene–drinking interaction was observed ($P_{interaction} = 9.7 \times 10^{-5}$ or 4.6×10^{-5}). For the *TERT* rs2853676 SNP, significant associations between the rs2853676 CT or TT genotype and GCA risk was found in all stratified analyses with age, sex, smoking and alcohol drinking status (all $P < 0.05$) (Table 4).

For the *VPS34* rs2162440 polymorphism, stratified analyses showed that significantly increased ORs for GCA development were only observed in the group aged 66 years or younger (OR 1.39, 95% CI 1.07–1.80, $P = 0.015$), males (OR 1.31, 95% CI 1.06–1.61, $P = 0.011$), nonsmokers (OR 1.41, 95% CI 1.12–1.79, $P = 0.004$), as well as nondrinkers (OR 1.48, 95% CI 1.18–1.87, $P = 0.001$). No statistically significant gene–environment interactions were observed between the *VPS34* SNP and the above-mentioned factors (all $P_{interaction} > 0.05$).

Discussion

Globally, gastric cancer incidence shows diverse characteristics by the two major topographical subsites, GCA, and noncardia gastric cancer [3]. GCA has distinct epidemiological, histopathological, and molecular characteristics which distinguish it from the adenocarcinomas of distal stomach [3–8]. Telomere shortening, in the course of somatic cell replication, ultimately results in replicative senescence and multiple aging-related complex genetic diseases. We previously found that shortened leukocyte telomere length significantly contributes to increased risk of GCA [10]. In humans, various genetic variations have been identified to be associated with leukocyte telomere length via GWAS [20–29] and most of these polymorphisms have been proved to confer cancer susceptibility. In the current study, we, for the first time, systematically evaluate the involvement of these telomere length-related genetic variations in GCA development. We found that *CXCR4* rs6430612, *TERT* rs10069690 and rs2853676, as well as *VPS34* rs2162440 polymorphisms significantly contribute to GCA risk in different Chinese populations.

CXCR4, the receptor of chemokine CXCL12, plays an important role in gastric cancer. Overexpressed CXCR4 was associated with more advanced tumor stage and poorer survival for gastric cancer patients [33]. CXCR4 can activate either the NF-κB/STAT3 signaling or the PI3K/AKT/mTOR signaling, while NF-κBp65 can then transcriptionally activate CXCR4 [33–35]. CXCR4-mediated invasion can be regulated by HER2, CD44, miR-139, and DARPP-32 in gastric cancer cells [36, 37]. CXCL12/CXCR4 signaling activates leukocytes and is often induced by proinflammatory stimuli, which can partially illuminate the association between rs6430612 and telomere length as well as GCA risk.

Human TERT is essential for the maintenance of telomere length, chromosomal stability, and cellular immortality. Accumulated evidences demonstrated that various *TERT* SNPs including rs10069690 and rs2853676 are associated with multiple cancer types [25, 38–44]. Duan et al. reported in a case–control study enrolled a total of 302 patients and 300 control individuals, and found that both rs10069690 and rs2853676 polymorphisms were significantly associated with gastric cancer [41]. However, they did not show the detailed information of gastric cancer patients whether or not including GCA. In the present study with 1024 GCA patients and 1118 controls, we, for the first time, demonstrated that a significantly increased GCA risk was associated with minor alleles of both SNPs (both $P < 10^{-5}$). For the detailed mechanistic insights into these GCA risk-associated SNPs, Killedar et al. found that the rs10069690 T allele creates an additional splice donor site in intron 4 of *TERT*, and results in co-production of full-length TERT and an alternatively spliced, INS1b, transcript [38]. INS1b protein does not have telomerase enzyme activity, but retains its ability to bind to the telomerase RNA subunit. This leads to decreased telomerase activity and telomere shortening [38]. These data are in line to our findings that the rs10069690 T allele is a GCA risk allele associated with short telomere length.

In a previous study, Mangino et al. conducted a GWAS of 314,075 SNPs and validated the results in a second cohort (n for both cohorts combined = 2790) and identified *VPS34* rs2162440 as a novel leukocyte telomere length-associated variants [27]. The involvement of rs2162440 in cancer development is unclear and we provided the first detailed data on its significant contribution to GCA risk. As a component of the PI3 kinase family, VPS34 regulates multiple aspect of the cell physiology [45]. Interestingly, Vps34, the VPS34 yeast orthologue, is directly involved in the pathway controlling telomere length variation [46]. As a result, it is possible that the *VPS34* rs2162440 may impact telomere maintenance, lead to an increased risk of genetic instability and therefore of tumorigenesis.

In all, to our knowledge, we, for the first time, identified four leukocyte telomere length-related genetic variants that significantly confer susceptibility to GCA. These results are consistent with our initial findings on association between leukocyte telomere length and GCA risk, further supporting the importance of telomere biology during carcinogenesis. However, some limitations exist in this case–control study.

For example, there might be inherent selection bias, since all GCA cases were recruited from hospitals. As a result, our findings warrant to be validated in a population-based prospective study in the future.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

Human rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent to be included in the study was obtained from all individuals.

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