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ORIGINAL ARTICLE

Two genetic variants in telomerase-associated protein 1 are associated with stomach cancer risk

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This study examined the impact of two single-nucleotide polymorphisms (SNPs) in the telomerase-associated protein 1 (TEP1) gene on the risk of breast, colorectal, hepatocellular, lung and stomach cancer. A significantly increased stomach cancer risk associated with the GG genotype at rs1760893 (odds ratio (OR) = 1.64, 95% confidence interval (CI) = 1.23–2.20, *P* = 0.004) or CC genotype at rs1713423 (OR = 2.40, 95% CI = 1.88–3.07, *P* < 0.0001) was observed, compared with their wild-type counterpart. The GG genotype at rs1760893 was also associated with enhanced hepatocellular cancer susceptibility (OR = 1.46, 95% CI = 1.05–2.03, *P* = 0.02). In classification and regression tree analysis, individuals carrying the CC genotype at rs1713423 had 2.69-fold increased risk of stomach cancer (95% CI = 2.18–3.32, *P* < 0.0001) compared with the TT and TC genotypes. The current results suggested that genetic variants at TEP1 SNPs rs1760893 and rs1713423 may be associated significantly with increased risk of stomach cancer.
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INTRODUCTION

Cancer is the leading cause of death worldwide. Lung, liver, stomach, colorectal and breast cancers cause the most cancer deaths each year.[1] Inherited genetic is one determinable factor of cancer causes, and the most common type of inherited genetic variant is the single-nucleotide polymorphism (SNP). SNP is a DNA sequence variation occurring within a population in which a single nucleotide in the genome differs between members of a biological species or paired chromosomes. Most commonly, SNPs are found in the DNA between genes, but when SNPs occur within a gene or in a regulatory region near a gene, they may have a role in disease by affecting the gene's function, resulting in an increased disease susceptibility or protection from disease.[2]

Telomerase-associated protein 1 (TEP1) is a component of the telomerase ribonucleoprotein complex and is thought to responsible for catalyzing the addition of new telomeres to chromosomes.[3] The human TEP1 gene resides on chromosome 14q11.2, and there are two isoforms of this protein, the isoform1 is 2627aa and the isoform2 is 2519aa. The isoform2 lacks exons 5 and 6 compared with the isoform1.[4] The mRNA expression of TEP1 is increased in tumor samples compared with normal breast tissue.[5] TEP1 protein is more abundant in higher-grade ovarian cancer.[6] TEP1 is also a component of the vault particle, a large cytoplasmic ribonucleoprotein complex consisting of major vault proteins, vault poly polymerase, small vault RNAs and TEP1.[7] TEP1 protein is required for vault RNA stability and its association with the vault particle.[8] Vaults are associated with the nuclear pore complex and have a role in the transportation of molecules, such as mRNA, from the nucleus to parts of the cytoplasm.[9] Vaults are overexpressed in cancer patients diagnosed with multidrug resistance and have been related with the resistance against many chemotherapy treatments.[10–12]

Several studies have analyzed the association of genetic variants in TEP1 with the susceptibility to certain cancers. For example, Chang *et al.*[13] revealed the significant correlation of four SNPs (rs2228041, rs2228026, rs1713418 and rs2297615) in the TEP1 gene with the increased risk of bladder cancer. Andrew *et al.*[14] observed that another TEP1 SNP (rs1760897) is associated with enhanced bladder cancer susceptibility. In addition, Jung *et al.*[15] studied the impact of telomere maintenance gene polymorphisms on hepatocellular carcinoma and reported that the SNP at rs1713449 in TEP1 is strongly associated with increased risk of hepatitis B virus-associated hepatocellular cancer development.

Recently, we carried out a genome-wide association study between SNPs and risk of stomach cancer in Korean population[16] and identified that two SNPs in the TEP1 gene (rs1760893, rs1713423) were correlated with high risk of this cancer. In this study, to further analyze haplotypes, to increase statistical power and to determine whether the two SNPs was also involved in other cancers, we compared the prevalence of genotypes at these two SNPs between a group of 3065 patients who had 5 different types of cancers (breast, colorectal, hepatocellular, lung, stomach) and a control group of 1375 individuals.

MATERIALS AND METHODS

Study populations

The population consisted of 1375 controls and 590 patients with breast cancer, 678 patients with colorectal cancer, 598 patients with hepatocellular carcinoma,

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299 patients with lung cancer and 900 patients with gastric cancer from the general population. They were used for the calculation of allele frequencies of the SNPs and for the construction of haplotypes. All cancer patients underwent curative surgical resection at the Samsung Medical Center, a major referral center for cancer in Seoul, Korea from 2003 to 2012.[2] Age- and sex-matched control participants for the five cancers were selected from among healthy individuals who visited the Samsung Medical Center for regular health checkups. Peripheral blood samples from all participants were collected according to ethical guidelines on the use of human tissues and biological samples in research after obtaining appropriate institutional review board permission and written informed consent from all participants. Information regarding demographics was obtained by using an interviewer-administered questionnaire.

Genotyping

Genomic DNA from peripheral blood lymphocytes was extracted using a QIAamp Blood Kit (Qiagen, Valencia, CA, USA). DNA quality was checked using an ultraviolet (UV) spectrophotometer (Pharmacia Biotech, Cambridge, UK) and the PicoGreen Double-Stranded DNA Quantitation Kit (Molecular Probes, Eugene, OR, USA) using a SpectraMax Gemini UV spectrometer (Molecular Devices, Sunnyvale, CA, USA). Genotyping was performed using the homogeneous MassExtend assay (Sequenom, San Diego, CA, USA), as described previously.[17] Both the amplification and extension primers were designed using the Sequenom Assay Design program. PCR and primer extension reactions were performed according to the protocol for hME procedure (MassARRAY System Training User's Guide; Sequenom).

Classification and regression tree (CART) analysis

CART analysis of the two SNPs (rs1760893, rs1713423) was performed using the stomach cancer and control samples.[18–20] The initial split was by rs1713423 and the second split was by rs1760893. Each node contains frequencies and percentages of case and controls. Subgroups of individuals with differential risk associations with stomach cancer were identified in the different terminal nodes of the tree. Odds ratios (ORs), 95% confidence intervals (CIs) and *P*-values were computed.

Table 1 Demographic distribution

	Age in years, mean ± s.d.	*Total*	*Men*	*Women*
Control	60 ± 14	1375	1072	303
Cancer				
Breast	49 ± 10	590		590
Colorectal	60 ± 11	678	560	118
Hepatocellular	53 ± 10	598	480	118
Lung	59 ± 11	299	226	73
Stomach	57 ± 12	900	626	274

*Breast cancer is female-matched.

Statistical analyses

The association between genotypes and the prevalence of each cancer was analyzed using Pearson chi-square test and Cochran–Armitage test for trend. Multivariate logistic regression analysis was performed using a web tool for SNP analysis (SNPStats, Barcelona, Spain) in both the patient and control groups.[21] ORs, 95% CIs and *P*-values were obtained. All *P*-values were two-tailed and a level <0.05 was considered statistically significant.

RESULTS

Subject characteristics

The study population comprised of 3065 cancer patients (breast cancer, $n=590$; colon cancer, $n=678$; hepatocellular cancer, $n=598$; lung cancer, $n=299$; and stomach cancer $n=900$) and 1375 healthy individuals (Table 1). Men accounted for about three times the number of women in both the control and patient groups. The mean age (± s.d.) was 49 ± 10 years in breast cancer, 60 ± 11 years in colorectal cancer, 53 ± 10 years in hepatocellular cancer, 59 ± 11 years in lung cancer, 57 ± 12 years in stomach cancer and 60 ± 14 years in the control group. The human TEP1 gene is located on chromosome 14q11.2 from base 20365667 to 20414172 in GRCh38 and has 11 transcript splice variants. The longest transcript length is 10694 bps and there are 54 exons (Figure 1). The rs1760893 SNP was identified in the promoter region and the rs1713423 in the twelfth intron of the TEP1 gene.

Genotypic distribution of the two SNPs in the five human cancers

Genotypic distributions at the two SNPs in the five cancer types were compared with the control group (Table 2). Genotypic distributions at rs1760893 and rs1713423 in breast, colorectal, hepatocellular and lung cancer were similar to that in the control group. But in patients with stomach cancer, the distribution differed significantly. The frequencies of the GG genotype at rs1760893 and the CC genotype at rs1713423 were significantly higher in the stomach cancer group than in the control group (rs1760893: 15% vs 10%, Chi-square $P=0.003$; rs1713423: 35% vs 19%, Chi-square $P<0.0001$).

The linkage disequilibrium structure of TEP1 SNPs in the populations of Han Chinese in Beijing and Japanese in Tokyo (CHB +JPT) was analyzed by Haploview[22] (Supplementary Figure S1). No strong combination between rs1760893 and rs1713423 was observed in CHB+JPT (D'-value: 0.15) and our Korean (D'-value: 0.20) populations. The haplotype distribution was similar between the Korean (control group) and CHB+JPT populations (Supplementary Table S1). However, haplotypes of CT and CG had 1.42-times (95% CI = 1.18–1.71; $P=0.0002$) and 1.81-times (95% CI = 1.52–2.15; $P<0.0001$) enhanced risk of stomach cancer compared with the haplotype TT.

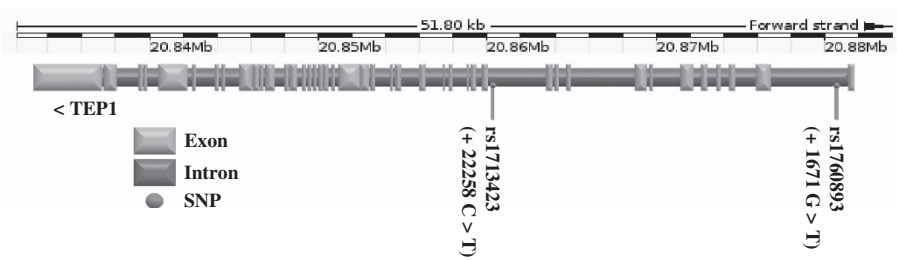

Figure 1 Location of the two single-nucleotide polymorphisms (SNPs) in the TEP1 gene is illustrated. The first base of the transcription start site is counted as position +1 (GenBank accession no. NC-000014.9). G → T indicates guanine to thymine, and C → T indicates cytosine to thymine. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 2 Association of individual SNP with the prevalence of cancers

		Cancers				
Genotype	*Control (%)*	*Breast (%)*	*Colorectal (%)*	*Hepatocellular (%)*	*Lung (%)*	*Stomach (%)*
SNP_1(rs1760893)						
GG	120 (10)	53 (10)	69 (9)	76 (14)	29 (10)	119 (15)
GT	512 (45)	220 (42)	385 (49)	238 (44)	133 (47)	353 (45)
TT	517 (45)	248 (48)	338 (43)	224 (42)	123 (43)	312 (40)
Total	1149	521	792	538	285	784
P[a]						
Chi-square[b]		0.61	0.16	0.08	0.81	0.003
Trend[c]		0.41	0.84	0.04	0.72	0.002
SNP_2(rs1713423)						
CC	210 (19)	40 (15)	158 (21)	113 (22)	8 (14)	289 (35)
TC	539 (48)	139 (51)	321 (44)	245 (48)	34 (60)	322 (39)
TT	372 (33)	92 (34)	256 (35)	153 (30)	15 (26)	213 (26)
Total	1121	271	735	511	57	824
P[a]						
Chi-square[b]		0.30	0.14	0.20	0.23	<0.0001
Trend[c]		0.32	0.74	0.08	0.82	<0.0001

Abbreviation: SNP, single-nucleotide polymorphism.
[a]*P*-values were based on the association between control and each cancer.
[b]Pearson chi-square test.
[c]Cochran–Armitage trend test.

Multivariate logistic regression analysis

Multivariate logistic regression analysis was performed to determine the effect of genotypic variants at rs1760893 and rs1713423 on the risk of the five cancers (Table 3). Genotypic variants at these two SNPs were not associated significantly with susceptibility of breast, colorectal and lung cancers. However, individuals who had the GG genotype at rs1760893 had a 1.46-times greater risk of hepatocellular cancer (codominant model, 95% CI = 1.05–2.03; $P = 0.02$) and a 1.64-times greater risk of stomach cancer. In addition, the CC genotype at rs1713423 was associated with a significantly increased risk of stomach cancer (codominant model, OR = 2.40; 95% CI = 1.88–3.07; $P < 0.0001$) compared with the TT genotype. The enhanced susceptibility was also observed in the dominant and recessive models (Supplementary Table S2).

CART analysis of the rs1760893 and rs1713423 SNPs in stomach cancer

The rs1760893 and rs1713423 SNPs that had been significantly associated with stomach cancer risk were further analyzed for their interaction in the CART analysis (Figure 2). The initial split was by rs1713423 because genetic variant at this SNP represented higher susceptibility than the SNP at rs1760893 (OR: 2.40 vs 1.64). Individuals who had the TT or TC genotype at rs1713423 represented 33.8% of the stomach cancers. However, individuals with the CC genotype at this region constituted 57.9% of the stomach cancers. Further split of individuals with the CC genotype at rs1713423 (Node2) into binary subgroups by rs1760893 determined that the percentage of patients with the TT or TG genotype at rs1760893 was 58% (Node3) and the percentage of patients with the GG genotype was 57.3% (Node4). There was no significant increase of patient proportion from node2 to node3 or node4. Table 4 summarizes the risk estimates for individuals in each node. Individuals who had the variant homozygote (CC genotype) at rs1713423 had a 2.69-times enhanced risk of stomach cancer (95% CI = 2.18–3.32; $P < 0.0001$) compared with individuals with the wild-type homozygote or heterozygote (TT or TC genotype). In addition, individuals who had a variant homozygote (CC genotype) at rs1713423 and a variant homozygote (GG genotype) at rs1760893 did not have a further increased risk of stomach cancer (OR: 2.69 vs 2.63), indicating no cumulative effect of the two SNPs on stomach cancer susceptibility.

Impact of genetic variant at rs1760893 or rs1713423 on TEP1 expression

The impact of genetic variant at rs1760893 or rs1713423 on TEP1 expression was analyzed by using a database portal The Genotype-Tissue Expression (GTEx) (http://gtexportal.org).[23,24] However, there was no data available for SNPs rs1760893 and rs1713423 in this database. We then determined SNPs that were near the rs1760893 or rs1713423 and correlated significantly with TEP1 expression in this database (Supplementary Figure S2A). In result, seven SNPs rs1760891, rs1713434, rs4246977, rs1713435, rs1760889, rs1760955 and rs1713428 near the rs1760893 were identified to associate significantly with the gene's expression. Linkage disequilibrium structure analysis revealed that rs1760893 has a strong recombination with the other SNPs in the populations of CHB+JPT and CEU+TSI (Northern and Western European and Toscans in Italy) (Supplementary Figures S2B and C). These results suggested that genetic variant at rs1760893 may be associated with the TEP1 expression.

DISCUSSION

In this study, the association of genetic variants at two SNPs (rs1760893, rs1713423) in the TEP1 gene with breast, colorectal, hepatocellular, lung and stomach cancer risk was investigated. The rs1760893 and rs1713423 SNPs correlated significantly with an increased risk of stomach and/or hepatocellular cancer, suggesting that genetic variants in these two regions may affect stomach and/or hepatocellular cancer susceptibility. SNPs occurring within a gene or

Table 3 Multivariate logistic regression analysis of individual SNP and cancer risk

	Codominant model[a]				*Log-additive model*	
	T/G		G/G			
Cancer	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
SNP_1(rs1760893)						
Breast	0.90 (0.72–1.11)	0.32	0.92 (0.64–1.32)	0.65	0.94 (0.80–1.10)	0.41
Colorectal	1.15 (0.95–1.39)	0.15	0.88 (0.63–1.22)	0.44	1.01 (0.88–1.17)	0.84
Hepatocellular	1.07 (0.86–1.34)	0.53	1.46 (1.05–2.03)	0.02	1.17 (1.00–1.36)	0.05
Lung	1.09 (0.83–1.44)	0.53	1.02 (0.65–1.59)	0.95	1.04 (0.85–1.26)	0.72
Stomach	1.14 (0.94–1.39)	0.18	1.64 (1.23–2.20)	0.004	1.24 (1.09–1.42)	0.002
SNP_2(rs1713423)						
Breast	1.04 (0.78–1.40)	0.78	0.77 (0.51–1.16)	0.21	0.91 (0.75–1.10)	0.32
Colorectal	0.87 (0.70–1.07)	0.18	1.09 (0.84–1.42)	0.50	1.02 (0.90–1.16)	0.74
Hepatocellular	1.11 (0.87–1.41)	0.42	1.31 (0.97–1.76)	0.08	1.14 (0.98–1.32)	0.08
Lung	1.56 (0.84–2.91)	0.16	0.94 (0.39–2.27)	0.90	1.04 (0.72–1.52)	0.82
Stomach	1.04 (0.84–1.30)	0.70	2.40 (1.88–3.07)	<0.0001	1.54 (1.36–1.74)	<0.0001

Abbreviations: CI, confident interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]TT was the reference in the codominant model.

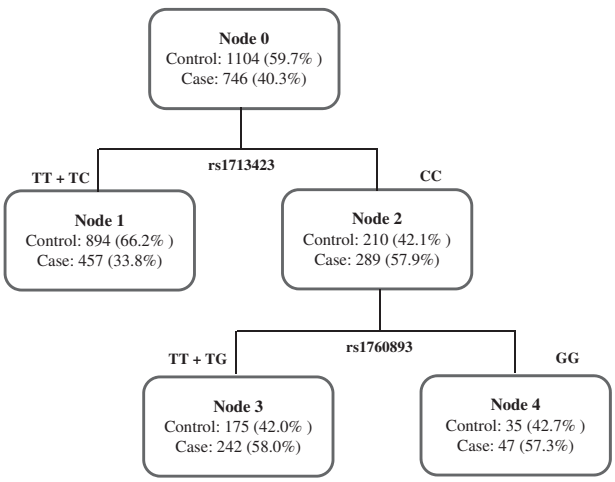

Figure 2 Tree structure of the classification and regression tree (CART) analysis of the two SNPs (rs1760893, rs1713423) in stomach cancer. The initial split was by rs1713423 and the second split was by rs1760893. Each node contains frequencies and the percentages of case and controls.

in a regulatory region near a gene may have a role in disease by affecting the gene's function. As rs1760893 is located at the promoter region of TEP1, this variation may regulate the gene's expression by altering transcriptional efficacy. Substantial differences in the transcriptional level result from genetic variants in the promoter regions of genes.[25,26] The rs1713423 SNP, which is located within the intron of TEP1, may affect splicing of the TEP1 transcript or disturb its expression by altering transcription factor-binding sites.

Although telomerase activity is associated with TEP1, this protein is not necessary for proper telomerase function and is not a component of the core telomerase complex.[27] Use of TEP1-deficient mice established that TEP1 is not essential for telomerase activity or telomere length maintenance *in vivo*.[28] However, TEP1, heat-shock protein 90 and topoisomerase IIα associate directly with bloom syndrome protein (BLM) helicase in immortalized cells using the alternative lengthening of telomere (ALT) pathway and modulate its helicase activity.[29] Cells deficient in BLM helicase display increased telomere association between homologous chromosomes. BLM helicase is important for proper telomere maintenance and function in ALT cells.[30] TEP1 can have a role in telomere maintenance in ALT cells. About 5–10% of human cancers rely on the ALT pathway to maintain telomere length.[31]

The expression of TEP1 is prevalent in multiple human tissues, including the lung, liver and stomach. In one study, TEP1 was expressed in 93% of 92 human lung cancer tissue samples and all 92 corresponding non-neoplastic lung tissues.[32] TEP1 was also reportedly expressed in all 23 human hepatocellular carcinoma samples and corresponding adjacent liver tissue.[33] In other studies, the positive rate of TEP1 mRNA from 24 human gastric cancer samples and corresponding non-cancerous tissues was 100% and 91.7%, respectively.[34] There was no significant difference of TEP1 expression between cancer and corresponding non-cancerous tissues in these three tissue types. TEP1 is known to exert its role through an interaction with molecules, such as major vault proteins,[7] small vault RNAs[7] and BLM.[29] Therefore, their expression levels are quite important for TEP1 function and may be associated with TEP1-related cancer risk. Expression levels of major vault proteins were high in the stomach and colon, medium in the lung and liver and low in the breast.[35] In contrast, BLM was high in the stomach, colon, lung and breast, but low in the liver.[35] Accordingly, it is likely that different SNPs in TEP1 may confer different susceptibility to cancer development according to the expression levels of the proteins in each tissue.

In conclusion, TEP1 genetic variants at rs1760893 and rs1713423 were presently associated significantly with stomach cancer susceptibility. We also speculate that the genetic variant at rs1760893 located at TEP1 promoter region may influence the gene's expression.

Table 4 Stomach cancer risk estimates of CART terminal nodes

Node	*Genotype*	*Control (%)*	*Case (%)*	*OR (95% CI)*	P-*value*
1	rs1713423 (W,H)	894 (66.2%)	457 (33.8%)	1 (Reference)	—
2	rs1713423 (V)	210 (42.1%)	289 (57.9%)	2.69 (2.18–3.32)	<0.0001
3	rs1713423(V)–rs1760893 (W,H)	175 (42.0%)	242 (58.0%)	2.71 (2.16–3.39)	<0.0001
4	rs1713423(V)–rs1760893 (V)	35 (42.7%)	47 (57.3%)	2.63 (1.67–4.13)	<0.0001

Abbreviations: CART, classification and regression tree; CI, confidence interval; H, heterozygote; OR, odds ratio; V, variant homozygote; W, wild-type homozygote.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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