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Original Article

Association analysis of selenoprotein S polymorphisms in Chinese Han with susceptibility to gastric cancer

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Abstract: Objective: selenoprotein S (*SelS*) gene polymorphism is closely related to a variety of malignant tumours. Here, we evaluate the association between *SelS* polymorphism and genetic susceptibility to gastric cancer. Methods: A case-control study was conducted to investigate the role of two *SelS* single nucleotide polymorphisms (SNPs) on the susceptibility to gastric cancer. The genotypes and genotype frequencies of the *SelS* were determined in 260 gastric cancer patients and 278 age-matched healthy controls. Polymerase chain reaction restriction fragment length of polymorphism (PCR-RFLP) was taken to genotype rs28665122 (G-105A) and rs34713741 (G-254A) within the *SelS* gene. The differences in the genotypic distribution between gastric cancer patients and healthy controls were analyzed with the Chi-square test for trends. Logistic-regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (CI), adjusting for age and sex. Results: For rs34713741 in *SelS*, the allele frequencies analysis indicated that the allele frequency of the T was higher in patients than in controls (P=0.001). There were significant differences of genotype frequencies and allele of rs34713741 polymorphism between gastric cancer group and control group (P<0.05). The relative risk of suffering from gastric cancer in T allele was 1.62 times of CC genotype in Hunan Han population (OR=1.62, 95% CI: 1.15~2.29). But there were no differences of genotype frequencies and allele of *SelS* rs28665122 polymorphism between gastric cancer group and control group. Conclusions: Allele T of *SelS* rs34713741 polymorphism is significantly associated with an increased risk of gastric cancer in Chinese population.

Keywords: Selenoprotein S, polymorphism, gastric cancer, case-control study

Introduction

Gastric cancer is the fourth most prevalent cancer and the second leading cause of cancer-related deaths worldwide, with 300,000 deaths in China each year. Surgery is the only means of curing the gastric cancer, however, 80% of the patients has been late gastric cancer when founded with higher distant metastasis rate, losing the chance of radical treatment. It is certain that gastric cancer is a genetic disease. The accumulation of multiple genetic changes, such as oncogene, tumor suppressor genes, DNA repair genes, cell adhesion molecules, telomerase, cell cycle regulatory factors and growth factors and so on, participates in the process of normal epithelial cells to cancerous. SNP can have functional effects on gene expression and ultimately protein activity either by causing amino acid changes in the protein or by influencing regulation of expression. The latter is the case for SNPs in gene promoter regions. Several functionally significant SNPs have been identified. Selenoprotein gene polymorphism is closely related to a variety of malignant tumours [1]. The biological functions of selenoprotein mainly including protecting cells from lipid hydroperoxides, participating in the inflammatory response and apoptosis endoplasmic reticulum associated protein degradation. Five types of selenoprotein have been studied, among which selenoprotein S is a protein widely expressed in a variety of tissues, located in the endoplasmic reticulum (ER) and in the plasma membrane [2, 3]. It protects cells from oxidative damage and apoptosis. It is involved in the control of the inflammatory response in the ER by retro-translocation of misfolded proteins from the ER lumen to cytosol leading to degradation through the protea-

Table 1. Distribution of gastric cancer and control groups

	Gastric cancer	Control	P
Age			
≥50	150	145	0.197
<50	110	133	
Gender			
Male	165	160	0.161
Femal	95	118	
Alcohol			
Yes	162	158	0.196
No	98	120	
Smoke			
Yes	145	148	0.565
No	115	130	

some. Shibata [13] indicated that the -105G>A promoter polymorphism of SEPS1 was associated with the intestinal type of gastric cancer. Individuals carrying A allele have higher risk of gastric cancer compared with GG genotype. However, the relationship between SelS gene polymorphism and the genetic susceptibility of gastric cancer have not been reported in Chinese people. The aim of the genotype distribution study was to evaluate the associations between SelS gene polymorphisms and gastric cancer in Chinese Han population of Hunan province.

Materials and methods

Patients and controls

The association between the SelS gene polymorphism and the risk for gastric cancer was assessed using a case-control study. A total of 538 subjects were evaluated. The gastric cancer group consisted 260 cases (79 males, 34 females, average age 54.2±11 years), collecting whole blood specimens from Hunan Cancer Hospital in October 2013 to October 2014 278 age- and sex-matched controls were healthy subjects from the third Xiangya Hospital of Central South University. Coronary heart disease, high blood pressure, diabetes, liver and kidney insufficiency were excluded. Research object were unrelated Han people in Hunan. There was no significant difference between the two groups of age, sex, drinking and smoking as shown in **Table 1**. All patients and healthy controls gave written informed consent, and the study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University.

DNA extraction

Peripheral venous blood samples (3 mL) were collected in vacutainer tubes containing the anticoagulant K2EDTA solution. The genomic DNA was isolated from peripheral blood leucocytes in accordance with the manufacturer's instructions using proteinase K and DNA extraction kits (OMEGA, America). DNA was stored at -20°C until use. The purity and concentration of DNA was determined by ultraviolet spectrophotometer.

Primer design and synthesis

The rs28665122 and rs34713741 of SelS gene on both sides of the gene sequences were acquired in Pubmed-SNP database respectively. The primers were designed by software premier 5.0. The upstream primer of rs28665122 was 5'-TCTTGGCGTTCCATGACC-3', and the downstream primer was 5'-AGCGTAGCCGGGATTTCTC-3'. The product length is 211 bp. The upstream primer of rs34713741 was 5'-CTTCCGGTGCGCTCCTAC-3' and the downstream primer was 5'-GGCGACCACTGACTTCCTT-3'. The length of product is 302 bp. The specificity of the primers were verified by the Primer-BLAST.

PCR amplification

The PCR reaction system is 25 μL, which including 2× Taq PCR Master Mix 12.5 μl, DNA template 2 μL (about 100 ng), Upstream and downstream primers 1 μL (10 μmol/L) respectively, Steam sterilization double water 8.5 μL. The rs28665122 of DNA was denatured at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 2 minutes. The rs34713741 of DNA was denatured at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The specificity and amplification efficiency of PCR products were detected in 2% agarose gel electrophoresis.

SNP genotyping

The rs28665122 was analyzed by FastDigest® Msc I (for 5'-TGG↓CCA-3' or 3'-ACC↑GGT-5')

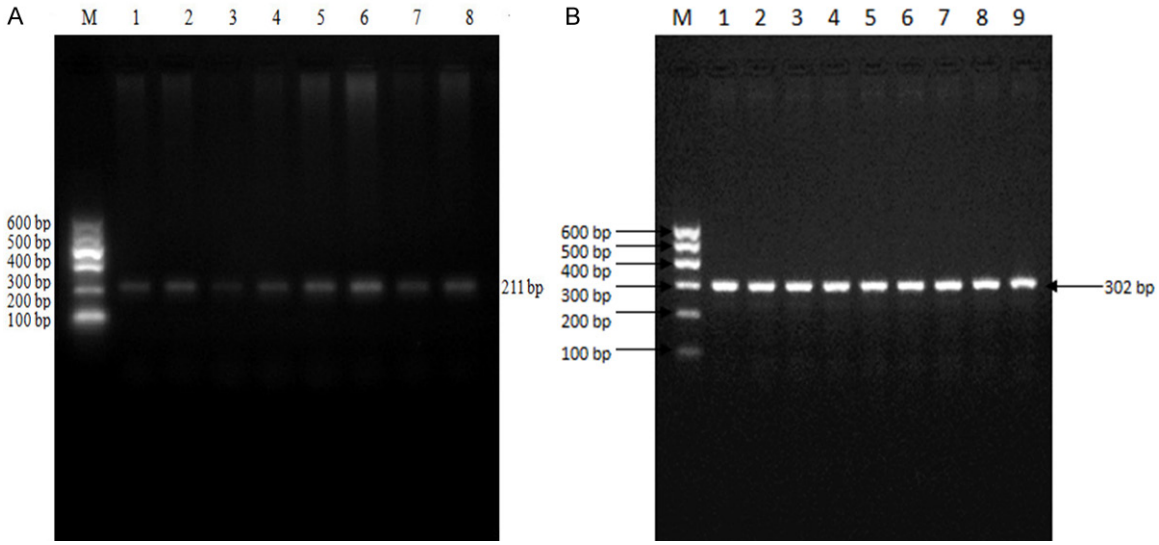

Figure 1. Electrophoresis results of SelS gene PCR products. M, DNA marker I.

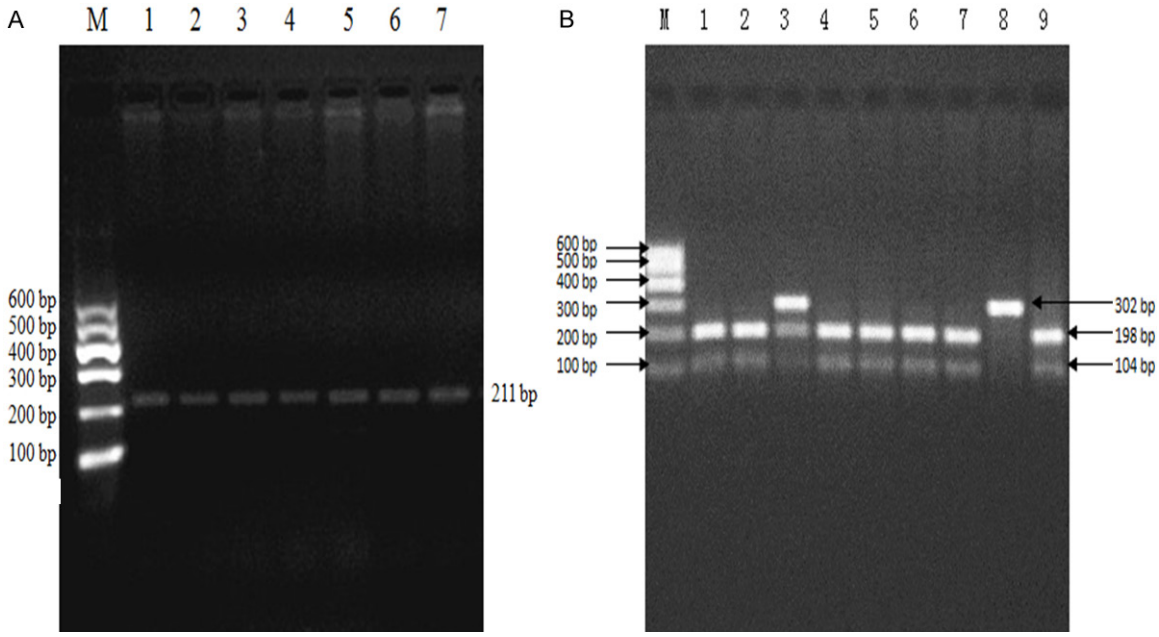

Figure 2. *SelS* rs28665122, rs34713741 loci gene polymorphism of enzyme products agarose gel electrophoresis results. M, DNA marker I; A. (1~7) are the GG genotype; B. 1, 2, 4, 5, 6, 7, 9 for CC genotype 1 and 3 for CT genotype, 8 for TT genotype.

digestion in a volume of 30 μL, including PCR product 10 μl, FastDigest®Msc I 1 μL, 10× Buffer 2 μL, Steam sterilization double water 17 μL. The mixture was rapidly centrifuged 5 s and bathed in 37°C water for 30 minutes. The rs34713741 was analysised by FastDigest® HinfI (for 5'-G↓ANTC-3' or 3'-CTNA↑G-5') digestion in a volume of 30 μL, including PCR product 10 μL, FastDigest® HinfI 1 μL, 10× Buffer 2 μL Steam sterilization double water 17 μL. The mixture was rapidly centrifuged 5 s and bathed in 37°C water for 5 minutes. Subsequently the digested products were analyzed on 2.5% agarose gels. Selecting 50 PCR products purified randomly and sequencing in Bo Shang biological technology co., LTD of Shanghai.

Statistical analysis

All analyses were performed using SPSS version 19.0 software package. A chi-square (χ^2) test was taken to evaluate the Hardy-Weinberg

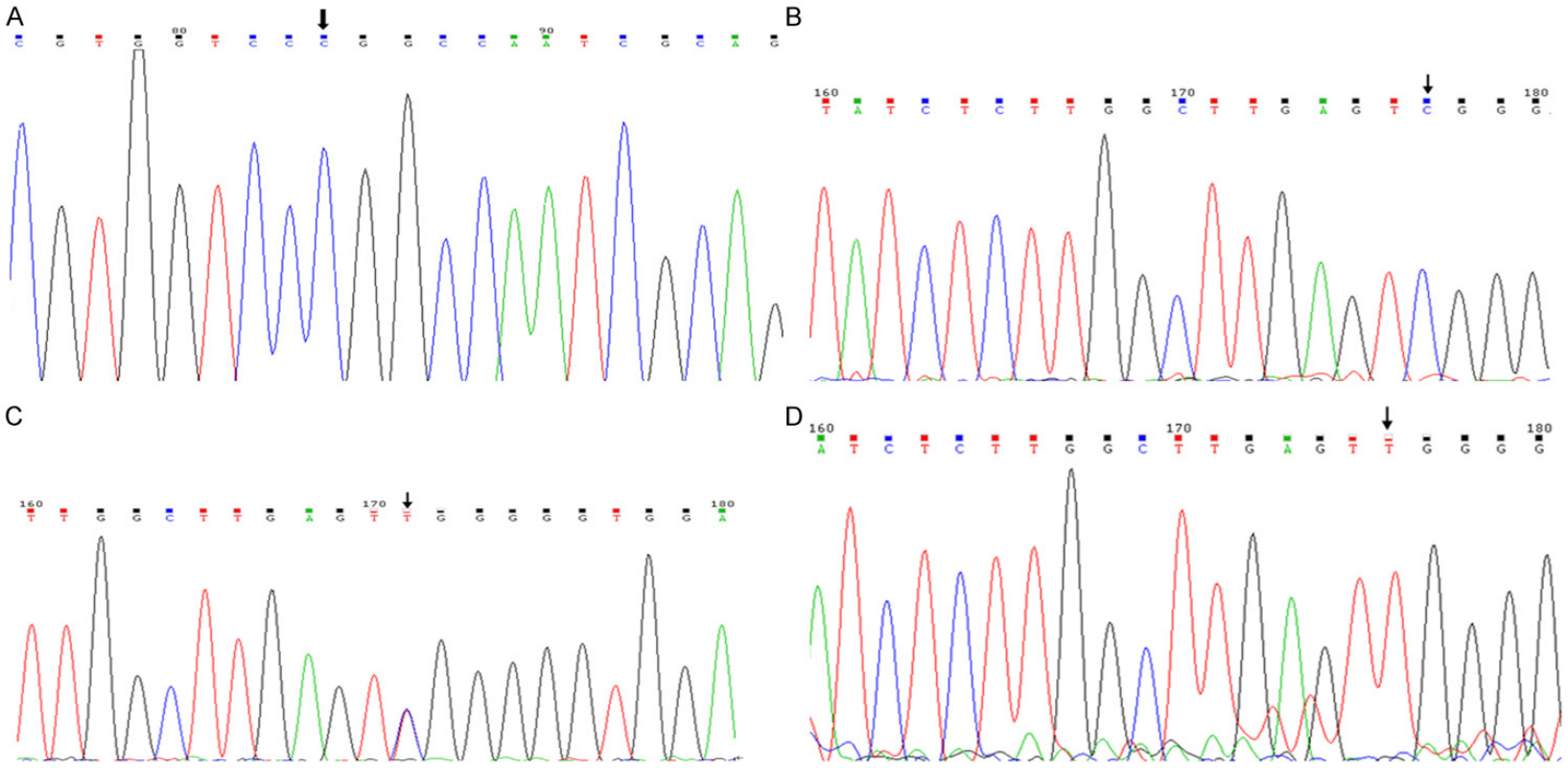

Figure 3. SelS rs28665122, rs34713741 loci PCR product sequencing results. ↓, The base mutation; A. GG type; B. CC type; C. CT type; D. TT type.

Table 2. SelS rs28665122 site genotype and allele frequency distribution in gastric cancer group and control group

	Genotype (%)			Allele frequency (%)	
Grouping	GG	GA	AA	G	A
Gastric cancer group (n=260)	100.0 (260/260)	0.0 (0/260)	0.0 (0/260)	100.0 (520/520)	0.0 (0/520)
Control group (n=278)	100.0 (278/278)	0.0 (0/278)	0.0 (0/278)	100.0 (556/556)	0.0 (0/556)

Table 3. SelS rs34713741 site genotype and allele frequency distribution in gastric cancer group and control group

	Genotype (%)			Allele frequency (%)		
Grouping	CC	CT	TT	C	T	$P_{H\text{-}W}$
Gastric cancer group (n=260)	50.4 (131/260)	38.8 (101/260)	10.8 (28/260)	69.8 (363/520)	30.2 (157/520)	0.206
Control group (n=278)	62.2 (173/278)	32.4 (90/278)	5.4 (15/278)	78.4 (436/556)	21.6 (120/520)	0.467
χ^2		7.671			10.419	
P		0.006			0.001	

equilibriums in cases and controls separately. The association between genetic polymorphisms and the risk of gastric cancer was estimated by conditional multiple logistical regression model, and the results were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). χ^2 test was used to analysis gastric cancer group and control group in allele frequency and genotype frequency distribution, a *P* value <0.05 was considered statistically significant.

Results

SelS gene amplification

As shown in **Figure 1**, A was SelS gene rs2866-5122 PCR products, product length was 211 bp. b was SelS gene rs34713741 PCR products, product length was 302 bp.

PCR-RFLP of the SelS genotypes

As shown in **Figure 2**, the polymorphism at position -105 of SelS had only one kind of result after Msc I digestion (A), namely the homozygous wild-type GG type. The electrophoregram only had a 211 bp banding. The polymorphism at position -254 of SelS had three kinds of results after digesting of HinfI, they were CC, TT and CT (B). Homozygous wild-type CC contained HinfI enzyme sites, producing 198 bp and 104 bp fragments, homozygous mutant TT did not contain HinfI enzyme sites, after HinfI digestion only had a 302 bp band. Heterozygous type CT was cut by HinfI into 302 bp, 198 bp and 104 bp pieces. PCR products were gone through DNA sequencing, the results were shown in **Figure 3**.

The genotype and allele frequency distribution

SelS rs28665122 site genotype and allele frequency distribution were shown in **Table 2**; SelS rs34713741 site genotype and allele frequency distribution were shown in **Table 3**. The study population had a group representative, which genotype distributions were accord with Hardy-Weinberg equilibrium ($P_{H\text{-}W}$>0.05). By chi-square test and unconditioned logistic regression analysis, the gastric cancer group and normal control group CC, CT, TT genotype comparison had significant difference (χ^2=7.671, P<0.05, OR= 1.62, 95% CI: 1.15~2.29); Two groups had significant difference in allele frequency (χ^2= 10.419, P<0.05).

The relationship of SelS gene rs34713741 polymorphism among age, gender and the clinical data. The rs34713741 CT and TT genotype were found in poorly differentiated carcinoma and lymph node group increased obviously after statistical analysis, had correlation with the pathological type and lymph node of gastric cancer group (P<0.05), but no correlation with age, sex, T-staging, Borrmann type, tumour location (P>0.05), as shown in **Table 4**.

Discussion

Selenoprotein S [4-11] (SelS) is a 189 amino acid trans-membrane protein. It has been proposed that SelS may function as a reductase, with the penultimate selenocysteine (Sec188) residue participating in a selenosulfide bond with cysteine (Cys174). SelS is widely expressed in a variety of tissues and has its characteristic expression. Selenoprotein S is a novel seleno-

Table 4. SelS rs34713741 genotype distribution in the various clinical factors of gastric cancer

Index	Genotype			*P*
	CC	CT	TT	
Age				
≥50	80	55	15	0.267
<50	51	46	13	
Gender				
Male	82	65	18	0.770
Female	49	36	10	
Differentiation				
High	47	30	3	0.440
Moderate	37	26	5	0.072
Poor	47	45	20	0.018
T-staging				
T1	21	13	5	0.639
T2	46	30	8	0.397
T3+T4	64	58	15	0.212
Lymph node				
Negative	60	33	7	0.014
Positive	71	68	21	
Borrmann				
B1	9	3	2	0.285
B2	51	49	12	0.174
B3	62	46	14	0.895
B4	9	3	0	0.081
Location				
Proximal	44	46	10	0.104
Distal	59	36	16	0.441
Total	28	19	2	0.294

protein located in the endoplasmic reticulum (ER) and the plasma membrane. It is involved in the control of the inflammatory response in and it protects cells from oxidative damage and apoptosis. It may affect the function of resisting tumors when SelS gene mutated. The 15 polymorphic sites of the SelS gene (539 delT, G105A, T12710C, G254A, G1393A, G3217A, G3705A, G4283A, A4502G, C5227T, A5265G, A6218G, T9707C, C1500A and C9000G) have been reported, but the G105A (rs28665122) and the G254A (rs34713741) were studied most. Curran [12] had tested 13 polymorphisms of the SelS gene except C-1500A and C9000G. The result showed that there were three polymorphisms associated with inflammation, G105A, G3705A and C5227T respectively. G105A site was functional site, which influenced the expression of inflammatory cytokines. Santos [10] examined 481 cases of hashimoto's thyroiditis patients and 516 healthy controls of SelS rs28665122, and found carrying A allele individuals compared with GG genotype individuals, had significantly increased the risk of hashimoto's thyroiditis. Shibata [13] indicated that the -105G>A promoter polymorphism of SEPS1 was associated with the intestinal type of gastric cancer, and individuals carring A allele had higher risk of gastric cancer compared with GG genotype.

This research adopts the PCR-RFLP technique of han nationality in hunan population genetic tests and verified by DNA sequencing enzyme result accuracy, confirm the crowd does not exist SelS rs28665122 gene polymorphism site, GG, GA, AA genotype frequency were 1.0, 0.0, 0.0, G, A allele frequency were 1.0, 0.0; and exist SelS rs34713741 gene polymorphism site, CC, CT, TT genotype frequency were 0.622, 0.324, 0.054, C, T allele frequency of 0.784, 0.216 respectively.

Taking together, in this case-control study, we have showed a significant association between SelS rs34713741 gene polymorphism and gastric cancer. In detailed clinicopathological analysis, gastric cancer are found significant associations between pathological type and lymph node, but no associations with age, sex, T-staging, Borrmann type, tumour location. We also have found that no differences of genotype frequencies and allele of SelS rs28665122 polymorphism between gastric cancer group and control group, which is different from Shibata's results. It is possible that the number of cases for this study is relatively low, and the results need to be validated in different populations and different subgroup in order to obtain more reliable results.

Disclosure of conflict of interest

None.

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