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Single-Nucleotide Polymorphism of the Exo1 Gene: Association with Gastric Cancer Susceptibility and Interaction with Smoking in Taiwan

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Abstract

Exonuclease 1 (Exo1) is an important nuclease involved in the mismatch repair system that contributes to the maintenance of genomic stability, modulation of DNA recombination and mediation of cell cycle arrest. Potential polymorphisms in Exo1 may alter cancer risks by influencing the repair activity of Exo1. We hypothesized that single-nucleotide polymorphisms (SNPs) in Exo1 might be associated with risks of gastric cancer. In this hospital-based study, the association of Exo1 A-1419G (rs3754093), C-908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P (rs9350) and C3114T (rs851797) polymorphisms with gastric cancer risk in a central Taiwanese population was investigated. In total, 179 patients with gastric cancer and 179 age- and gender-matched healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped. A significantly different distribution was found in the frequency of the Exo1 K589E genotype, but not the other genotypes, between the gastric cancer and control groups. The A allele Exo1 K589E conferred a significant (P = 0.0094) increased risk of gastric cancer. Gene-environment interactions with smoking were significant for Exo1 K589E polymorphism, which showed that the Exo1 K589E AG/AA genotype in association with smoking conferred an increased risk of 2.07-fold (95% confidence interval = 1.22-3.50) for gastric cancer. Our results provide the first evidence that the A allele of the Exo1 K589E may be associated with the development of gastric cancer and may be a novel and useful marker for primary prevention and anticancer intervention.

Key Words: Exo1, polymorphism, gastric cancer, carcinogenesis

Introduction

Gastric cancer is the fourth most common cancer world-wide and affects approximately 900,000 individuals every year (28). Although the identification of *Helicobacter pylori* has revolutionized the under-

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standing of its epidemiology and pathogenesis, the initiation etiology and genomic contributing factors of gastric cancer are still largely unknown (10). Apparently, both environmental and genetic factors are involved in gastric carcinogenesis. For example, tobacco smoking was recently included in the list of environmental factors that increase the risk of gastric cancer (33, 35) after low fruit and vegetables intake, high salt consumption (19, 27) and *H. pylori* infection (9). A meta-analysis was published showing that a 44% increase in the risk of gastric cancer among smokers compared to non-smokers (33). In addition, a systematic review and meta-analysis published in 2006 showed that a significant 79% and 22% increased risk of gastric cancer in male and female smokers, respectively (23). Furthermore, polymorphisms such as *CDH1* C-160A interacted with smoking to increase gastric cancer risk in smokers but not in non-smokers (21). However, it is commonly recognized that single environmental factor can only explain a small population of subject that develop gastric cancer. Thereafter, genetic factors may be more comprehensive and important. The responses of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are essential in preventing tumor initiation and progression. Mutations or defects in the DNA repairing system are essential for tumorigenesis (36). It is, therefore, logical to suspect that some genetic variants of DNA repair genes, such as exonuclease 1 (Exo1), might contribute to gastric cancer pathogenesis.

Sequence variants in DNA repair genes are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (11). Since single-nucleotide polymorphism (SNP) is the most frequent and subtle genetic variation in the human genome and has great potentials for application in association studies of complex diseases (16). DNA damages and genome instability have been thought as the first step of carcinogenesis. The DNA repair system is responsible for removing DNA damages and maintaining genome stability, and each type of DNA injury was repaired *via* its specific repair pathway. One of the major DNA repair pathways in human cells is the mismatch repair (MMR) which maintains genomic stability, modulates DNA recombination and mediates cell cycle arrest (12). This system is important in preventing malignancies and previous reports have indicated that deficient mutations of the mismatch repair system will lead to carcinogenesis including lung cancer (17, 37, 42). The exonuclease 1 gene (Exo1; MIM #606063) is a member of the MMR system and also belongs to the RAD2 nuclease family. It is located at chromosome 1q42-q43 and contains one untranslated exon followed by 13 coding exons and encodes a 846-amino acid protein (25, 31, 39). Exo1 can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells and with MSH3 in human cells (13, 24-26, 30, 32). Recent findings have indicated that mammalian Exo1 is responsible for mutation prevention and is essential for normal meiosis. They have also indicated mice with Exo1 inactivation predisposition have reduced survival time and increased risk in tumors development, specifically lymphoma (32).

Single-nucleotide polymorphisms (SNPs) of DNA repair genes have been reported to be associated with susceptibility to several cancers including oral, breast, gastric, prostate and colorectal cancers (1-8, 14, 34, 38, 40). These reports indicate that SNPs of the DNA repair system may affect gene function or expression level, and the capacity of gene-related systems may also be affected. Therefore, cancer susceptibility would be higher in people who carry high-risk genotypes. Several SNPs of Exo1 have been reported as the genetic risk factors of cancer. In 2005, a study investigating a Japanese population found that two polymorphisms of the Exo1 gene, T439M and P757L, were associated with colorectal cancer risk (43). In 2008, association between SNPs of Exo1 and lung cancer susceptibility was also examined in a Chinese population and the results indicate that K589E is associated with lung cancer risk (15). In order to understand and prevent local gastric cancer, we have chosen nine SNPs of Exo1, A1419G (rs3754093), C908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P (rs9350) and C3114T (rs851797), and investigated their frequencies in a Taiwanese population.

Materials and Methods

Study Population and Sample Collection

One hundred and seventy-nine cancer patients diagnosed with gastric cancer were recruited at the outpatient clinics of general surgery between 2005-2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients including histological details were all graded and defined by expert surgeons. All patients participated voluntarily, completed a self-administered questionnaire and provided peripheral blood samples. Equal number of non-cancer healthy volunteers used as controls were selected by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from known or unknown origin, and

Table 1. Characteristics of gastric cancer patients and controls

Characteristics	Controls (n = 358)			Patients (n = 358)			P^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (y)			62.1 (9.5)			63.8 (11.4)	0.58
Gender							0.36
Male	121	67.6		129	72.1		
Female	58	32.4		50	27.9		
Habit							
Cigarette smokers	117	65.4		128	71.5		0.21
Non-smokers	62	34.6		51	28.5		

[a]*P* based on two-sided Chi-square test without Yate's correction.

any familial or genetic diseases. Both groups finished a short questionnaire which included some indulgences. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping Assays

Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (20-25). Briefly, the following primers were used for

Exo1 A-1419G: 5'-AACTGACAGGCACACTTAAG-3' and 5'-GTAGAGAAGCCTTCTTACAC-3';
for Exo1 C-908G: 5'-GTTAGGTCTACCATAGCCTT-3' and 5'-TTCATGGTCACTTGTGGCTA-3';
for Exo1 A238G: 5'-AGTCTCTTACCTCTCAGATG-3' and 5'-TACATGCAATCTCTCCACCT-3';
for Exo1 C498T: 5'-AGCGTAGTAAGAATGGCTGA-3' and 5'-GATAAGAGAGCAGACGATTC-3';
for Exo1 K589E: 5'-GACACAGATGTAGCACGTAA-3' and 5'-CTGCGACACATCAGACATAT-3';
for Exo1 G670E: 5'-AATATGTCTGATGTGTCGCA-3' and 5'-TAGCTCGTCATTCACATGTA-3';
for Exo1 C723R: 5'-ACACCTACAGTCAAGCATAA-3' and 5'-ACTCTAGGAATCTGATTGCA-3';
for Exo1 L757P: 5'-CAGAATGGTCTTAAAATGGGTGT-3' and 5'-TTCAGAATAAGAAACAAGGCAAC-3';
and for Exo1 C3114T: 5'-CTACTTGACAACATTACAGA-3'and 5'-GAGAACCTGATTGTGTTATA-3'.

The following cycling conditions were performed: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. The PCR products were studied after digestion with EcoP15 I, HpyCH4 IV, Dpn II, Stu I, Mse I, Ear I, HpyCH4 IV, Mnl I, and Mse I, restriction enzymes for A1419G (cut from 386 bp A type into 144 + 242 bp G type), C908G (cut from 470 bp G type into 225 + 245 bp C type), A238G (cut from 367 bp G type into 178 + 189 bp A type), C498T (cut from 323 bp T type into 150 + 173 bp C type), K589E (cut from 306 bp G type into 110 + 196 bp A type), G670E (cut from 273 bp G type into 71 + 202 bp A type), C723R (cut from 264 bp A type into 66 + 198 bp G type), L757P (cut from 255 bp T type into 102 + 153 bp C type) and C3114T (cut from 602 bp C type into 173 + 429 bp T type), respectively.

Statistical Analyses

Only those matches with all the SNPs data (case/control = 358/358) were selected for final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of Exo1 SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's two-sided χ^2 test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the Exo1 genotypes between cases and controls. Data were recognized as significant when the statistical *P* was less than 0.05.

Results

The frequency distributions of selected characteristics of 179 gastric cancer patients and controls are shown in Table 1. Characteristics of patients and controls are all well matched. The mean age of the gastric cancer patients and the controls were 63.8 (standard deviation, SD = 11.4) and 62.1 (SD = 9.5) years, respectively. The ratio of male patients and controls is 72.1% and 67.6%, respectively. The ratio of cigarette smoker in patients and controls is 71.5% and 65.4%, respectively. All differences between both groups were no statistically significant (*P* >

Table 2. Distribution of Exo1 genotypes among gastric cancer patients and controls

Genotype	Controls	%	Patients	%	P^a
A1419G rs3754093					0.5857
AA	75	41.9	68	38.0	
AG	82	45.8	83	46.4	
GG	22	12.3	28	15.6	
C908G rs10802996					0.7788
CC	102	57.0	100	55.8	
CG	61	34.1	59	33.0	
GG	16	8.9	20	11.2	
A238G rs1776177					0.7483
AA	82	45.8	80	44.7	
AG	84	46.9	82	45.8	
GG	13	7.3	17	9.5	
C498T rs1635517					0.5655
CC	8	4.5	11	6.2	
CT	59	33.0	65	36.3	
TT	112	62.5	103	57.5	
K589E rs1047840					**0.0302**
AA	5	2.8	12	6.7	
AG	49	27.4	64	35.8	
GG	125	69.8	103	57.5	
G670E rs1776148					0.8869
AA	8	4.5	9	5.0	
AG	36	20.1	39	21.8	
GG	135	75.4	131	73.2	
C723R rs1635498					0.8065[b]
AA	137	76.5	132	73.8	
AG	39	21.8	43	24.0	
GG	3	1.7	4	2.2	
L757P rs9350					0.7672
CC	56	31.3	62	34.6	
CT	84	46.6	78	43.6	
TT	39	22.1	39	21.8	
C3114T rs851797					0.9465
CC	36	20.1	38	21.2	
CT	90	50.3	87	48.6	
TT	53	29.6	54	30.2	

[a]*P* based on two-sided Chi-square test without Yate's correction.
[b]*P* based on Fisher's exact test.

0.05) (Table 1).

The frequency of the genotypes for the Exo1 A1419G, C908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T between controls and the gastric cancer patients is shown in Table 2. Genotype distribution of various genetic polymorphisms of Exo1 K589E was significantly different between gastric cancer and control groups ($P = 0.0302$) while that for all the other polymorphisms was not significant ($P > 0.05$) (Table 2). To sum up, the Exo1 K589E is associated with higher susceptibility for gastric cancer. Representative PCR-based restriction analyses for the Exo1 K589E polymorphisms are shown in Fig. 1.

The frequency of the alleles for the Exo1 A1419G, Exo1 C908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T between controls and the gastric cancer patients is shown in Table 3. The allele frequency distributions of the Exo1 K589E showed that the A allele of Exo1 K589E is associated with higher susceptibility for gastric cancer while others are not (Table 3).

Table 3. Distribution of Exo1 alleles among gastric cancer patients and controls

Allele	Controls	%	Patients	%	P^a
A1419G rs3754093					0.3143
Allele A	232	64.8	219	61.2	
Allele G	126	35.2	139	38.8	
C908G rs10802996					0.6127
Allele C	265	74.0	517	72.3	
Allele G	93	26.0	199	27.2	
A238G rs1776177					0.6295
Allele A	248	69.3	482	67.6	
Allele G	110	30.7	234	32.4	
C498T rs1635517					0.2838
Allele C	75	20.9	174	24.3	
Allele T	283	79.1	542	75.7	
K589E rs1047840					**0.0094**
Allele A	59	16.5	163	24.3	
Allele G	299	83.5	553	75.7	
G670E rs1776148					0.6030
Allele A	52	14.5	114	15.9	
Allele G	306	85.5	602	84.1	
C723R rs1635498					0.5105
Allele A	313	87.4	615	85.8	
Allele G	45	12.6	101	14.2	
L757P rs9350					0.6518
Allele C	196	54.7	404	56.4	
Allele T	162	45.3	312	43.6	
C3114T rs851797					0.9402
Allele C	162	45.3	325	45.5	
Allele T	196	54.7	391	54.5	

[a]*P* based on two-sided Chi-square test without Yate's correction.

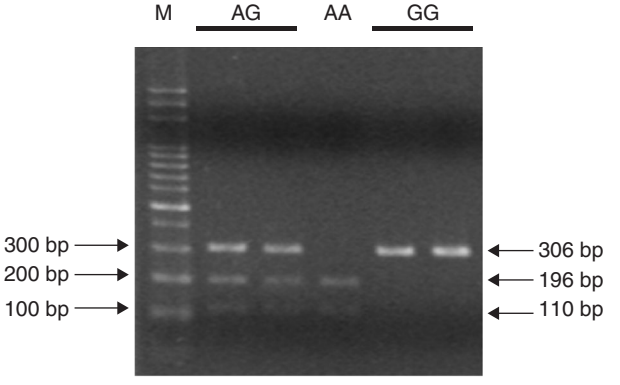

Fig. 1. PCR-based restriction analysis of the Exo1 K589E rs1047840 polymorphism shown by 2.5% agarose electrophoresis. M: 100 bp DNA size marker, G/G: enzyme indigestible homozygote, A/G: heterozygote, and A/A: enzyme digestible homozygote.

The genotype distribution of various genetic polymorphisms of Exo1 K589E was significantly different between the gastric cancer and the control groups who smoked (P = 0.0065) (Table 4) while those for the other SNPs were not significant (P > 0.05) (data not shown). In detail, distributions of Exo1 K589E A homozygote/heterozygote and G homozygote in controls and gastric cancer patients who were smoker were 35/82 and 60/68, respectively (P = 0.0065, OR = 2.07, 95% CI, 1.22-3.50) (Table 4). Distributions of Exo1 K589E A homozygote/heterozygote and G homozygote in controls and gastric cancer patients who were non-smokers were 19/43 and 16/35, respectively (P = 0.9337, OR = 1.03, 95% CI, 0.46-2.30) (Table 4).

Discussion

In order to determine potential biomarkers of gastric cancer, we selected up to nine SNPs of the Exo1 gene in this study and investigated the associations with the susceptibility of gastric cancer in a population in central Taiwan. Among the nine polymorphisms investigated, we found that variant genotypes of Exo1 K589E were significantly associated with a higher susceptibility of gastric cancer (Tables 2 and 3).

Table 4. Exo1 K589E rs1047840 genotype and gastric cancer after stratified by smoking

Variables	Exo1 K589E rs1047840 genotypes			
	GG (%)	AA + AG (%)	P^a	OR (95% CI)[b]
Smokers			**0.0065**[c]	
Controls	82 (70.1%)	35 (29.9%)		1.00
Patients	68 (53.1%)	60 (46.9%)		**2.07 (1.22-3.50)**[c]
Non-smokers			0.9337	
Controls	43 (69.4%)	19 (30.6%)		1.00
Patients	35 (68.6%)	16 (31.4%)		1.03 (0.46-2.30)

[a]*P* based on two-sided Chi-square test without Yate's correction.
[b]The ORs were estimated with multivariate logistic regression analysis.
[c]Statistically identified as significant.

Among the DNA repair system, one of the major roles is the MMR system which is responsible for correcting mismatches between bases and small insertion/deletion loops (20, 22). Exo1 is the only exonuclease involved in the human MMR system, playing a critical role as both 5'-3' and 3'-5' nucleases and contributing to the overall integrity of the MMR complex (18). Because the Exo1 plays a distinctive role in the MMR system, the Exo1 gene has become a famous target gene and is widely investigated for its association with risk of various malignants (15, 29, 41).

In this paper, we found that Exo1 K589E was associated with gastric cancer susceptibility in a central Taiwanese population, and the only polymorphism which has positive association is located on the 12exon of the Exo1 gene and its change causes the 589th amino acid of the Exo1 protein product from lysine to glutamic acid. The amino acid change at codon 589 might influence the products of the Exo1 mRNA for K589E is located at an exonic splicing enhancer (ESE) region (15). Our results in Taiwan are consistent with the work in Mainland China, which is also a sub-population of the Han-nationality, in an investigation of the association of Exo1 polymorphisms with lung cancer (15). On the contrary, Zienolddiny *et al*. have found no significant association of Exo1 K589E polymorphism and risk of non-small cell gastric cancer in a Caucasian Norwegian population (44). The reasonable explanation is that the similarity between ours and Jin's may be due to different ethnics; this polymorphism may be associated with Mongolian gastric cancer, but not in Caucasians.

Since smoking may be an environmental factor for gastric cancer (21), we have further analyzed the association between K589E genotype and gastric cancer risk in patients and controls who have cigarette smoking habits. Interestingly, the interaction between Exo1 K589E and cigarette smoking habit is obvious: subjects with the AA or AG genotype have a 2.07-fold higher risk of the gastric cancer than subjects with the GG genotype (Table 4). We propose that the A allele of K589E may affect the Exo1 activity slightly influencing its normal function. As those people with the A allele(s) are getting older, the alteration towards carcinogens may accumulated *via* continuous accumulation of the amounts of unremoved DNA adducts. Cigarette smoking, a well-known origin of DNA damage, releases many DNA damage inducers to our respiratory system and causes DNA damages to the cells. Therefore, if people who have high-risk genetic variant, such as the A allele of K589E, and also smoking habits, the combined effect of genetic and environmental factors would synergistically increase their gastric cancer susceptibilities. The present study is the most comprehensive assessment of the effects of genetic-smoking interaction on gastric cancer, adding to previous knowledge an updated and clearer understanding of the factors contributing to the heterogeneity of gastric cancer. Our results show that smoking is indeed a behavioral factor for gastric cancer and has synergistic effects with genetic factors.

In conclusion, this is the first study which focuses on the SNPs of Exo1 and gastric cancer in Taiwan, and the presence of the A allele of K589E is found to be associated with a higher risk of gastric cancer. The A allele of K589E may be a useful marker in gastric oncology for anticancer application and early cancer detection.

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