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

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Running head: EXO1 POLYMORPHISMS IN GASTRIC CANCER

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Abstract

Exonuclease 1 (Exo1) is an important nucleases involved in mismatch repair system that contributes to maintain genomic stability, to modulate DNA recombination, and to mediate cell cycle arrest. Potentially polymorphisms in *Exo1* may alter cancer risks by influencing the repair activity of Exo1. Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in *Exo1* were associated with risk 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 useful marker for primary prevention and anticancer intervention.

Key words: EXO1, polymorphism, gastric cancer, carcinogenesis

Introduction

Gastric cancer is the fourth most common cancer over the world and affects approximately 900,000 individuals every year (28). Although the identification of *Helicobacter pylori* has revolutionized the understanding of its epidemiology and pathogenesis, the initiation etiology and genomic contributing factors of gastric cancer are still largely unknown (9). 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 (12,33), after low fruit and vegetables intake, high salt consumption (20,39) and *H. pylori* infection (11). A meta-analysis was published, showing that a 44% increase in the risk of gastric cancer among ever smokers compared to never 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 (24). Furthermore, polymorphisms such as *CDH1* C-160A interacted with smoking to increase gastric cancer risk in smokers but not in never smokers (22).However, it is commonly recognized that single environmental factor can only explain a small part of subjects developed gastric cancer. Thereafter, the genetic factors may be more comprehensive and less ignorable. 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 (35). It is therefore logical to suspect that some genetic variants of DNA repair genes, such as exonuclease I (*ExoI*), might contribute to gastric cancer pathogenesis.

Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk (10). Since single-nucleotide polymorphism (SNP) is the most frequent and subtle genetic variation in the human genome and has great potential for application to association studies of complex disease (17). The DNA damages and genome instability have been thought as the first step of various carcinogenesis. The DNA repair system is responsible to remove DNA damage and maintaining the 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 maintaining genomic stability, modulating DNA recombination, and mediating cell cycle arrest (13). This system is important in preventing malignancies, and former reports indicated the deficient mutations of mismatch repair system will lead to various carcinogenesis, including lung cancer (18, 36, 42). The gene *exonuclease 1* (*Exo1*; MIM #606063) is a member which belongs to the MMR system, and also belong to the RAD2 nuclease family. It located at chromosome 1q42-q43, contains one untranslated exon followed by 13 coding exons and encodes an 846 amino acid

protein (26,31,38). Exo1 can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells and with MSH3 in human cells (14,25-27,30,32). Recent findings indicated that mammalian Exo1 is responsible in mutation prevention and it is essential for normal meiosis. They also indicated the mice with Exo1 inactivation predispose have reduced survival time and increased risk in tumors development, specifically lymphoma (32).

Single nucleotide polymorphisms (SNPs) of DNA repair genes have been reported associate with susceptibility to several cancers, including oral, breast, gastric, prostate, and colorectal cancers (1-8,34,37,40). These reports indicated the SNPs of DNA repair system may affect the gene's function or expression level, and the capacity of those genes related system will also affected. Therefore, the cancer susceptibility will higher in people who carry those risky genotypes. There are already several SNPs of *Exo1* have been reported as the genetic risk factors of cancer. In 2005, a study investigating Japanese population found that two polymorphisms of *Exo1* gene, T439M and P757L, are associated with colorectal cancer risk (43). In 2008, the association between SNPs of *Exo1* and lung cancer susceptibility was also examined in a Chinese population, indicating the K589E is associated with lung cancer risk (16). In order to understand and prevent local lung cancer, we have chosen up to nine SNPs of *Exo1*, A-1419G (rs3754093), C-908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P

(rs9350) and C3114T (rs851797), and investigated their frequencies in 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 patients include histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Equal number of non-cancer healthy volunteers 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 control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups finished a short questionnaire which included some indulgences and they were recorded. 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 (21-26). 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^{o}C for 5 min; 35 cycles of 94^{o}C for 30 s, 55^{o}C for 30 s, and 72^{o}C for 30 s; and a final extension at 72^{o}C for 10 min. The PCR products were studied after digestion with EcoP15 Ⅰ, HpyCH4Ⅳ, Dpn Ⅱ, Stu Ⅰ, Mse Ⅰ, Ear Ⅰ, HpyCH4Ⅳ, Mnl Ⅰ, and Mse Ⅰ, restriction enzymes for A-1419G (cut from 386 bp A type into 144+242 bp G type), C-908G (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 SNPs data (case/control =358/358) were selected into final analyzing. 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 was 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 were shown in Table I. These 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 in 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 of these differences between both groups were no statistically significant ($P > 0.05$) (Table I).

The frequency of the genotypes for the *Exo1* A-1419G, C-908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T, between controls and gastric cancer patients is shown in Table II. Genotype distribution of various genetic polymorphisms of *Exo1* K589E was significantly different between gastric cancer and control groups ($P = 0.0302$), while those for all the other polymorphisms were not significant ($P > 0.05$) (Table II). To sum up, the *Exo1* K589E is associated with higher susceptibility for gastric cancer. The representative PCR-based restriction analyses for the *Exo1* K589E polymorphisms were shown in Figure 1.

The frequency of the alleles for the *Exo1* A-1419G, *Exo1* C-908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T, between controls and gastric cancer patients is shown in Table III. The allele frequency distributions of the *Exo1*

K589E showed that A allele of *Exo1* K589E is associated with higher susceptibility for gastric cancer, while others are not (Table III).

Genotype distribution of various genetic polymorphisms of *Exo1* K589E was significantly different between gastric cancer and control groups who have smoking habit ($P = 0.0065$) (Table IV), 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 with smoking habit were 35/82 and 60/68, respectively ($P = 0.0065$, OR = 2.07, 95%CI, 1.22-3.50) (Table IV). Distributions of *Exo1* K589E A homozygote/heterozygote and G homozygote in controls and gastric cancer patients who with non-smoking habit were 19/43 and 16/35, respectively ($P = 0.9337$, OR = 1.03, 95% CI, 0.46-2.30) (Table IV).

Discussion

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

Among the DNA repair system, one of the major roles is the MMR system, which is responsible to correct the mismatch between bases and the small insertion/deletion loops (21,23). 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 (19). Because the Exo1 plays a distinctive role in the MMR system, the *Exo1* gene has become a famous target gene and widely investigated for its association with risk of various malignants (15,29,41).

In this paper, we have found that *Exo1* K589E was associated with gastric cancer susceptibility in central Taiwan, and the only one polymorphism which has positive association locates on the exon12 of *Exo1* gene and its change will cause the 589th amino acid of Exo1 protein product from lysine to glutamic acid. The amino acid change at codon 589 might influence the products of *Exo1* mRNA, for K589E was located at an exonic splicing enhancer (ESE) region (16). Our results in Taiwan are

consistent with the work in Mainland China, which is also a subpopulation of the Han-nationality, investigation the association of Exo1 polymorphisms with lung cancer (16). 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 (29). The reasonable explanation is that the similarity between ours and Jin's may be caused by ethnic; this polymorphism may associate with Mongolian gastric cancer, but not Caucasians'.

Since smoking may be an environmental factor for gastric cancer (22), 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, people who with the AA or AG genotype have a higher risk of the gastric cancer in 2.07-fold than people who with GG (Table IV). We propose that the A allele of K589E may affect the Exo1 activity, slightly influence its normal function. As those people with A allele(s) getting older, the alteration towards carcinogens may accumulated via the amounts of unremoved DNA adducts keep on rising. Cigarette smoking, a well-known origin of DNA damage, will release many DNA damage inducers to our respiratory system and cause DNA damages to the cells. Therefore, if people who have risky genetic variant, such as the A allele of K589E, and also have the smoking habit, the joint effect of genetic and environmental factors will 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 the behavioral factor for gastric cancer, and have 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 was 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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Figure Captions

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

Figure 1

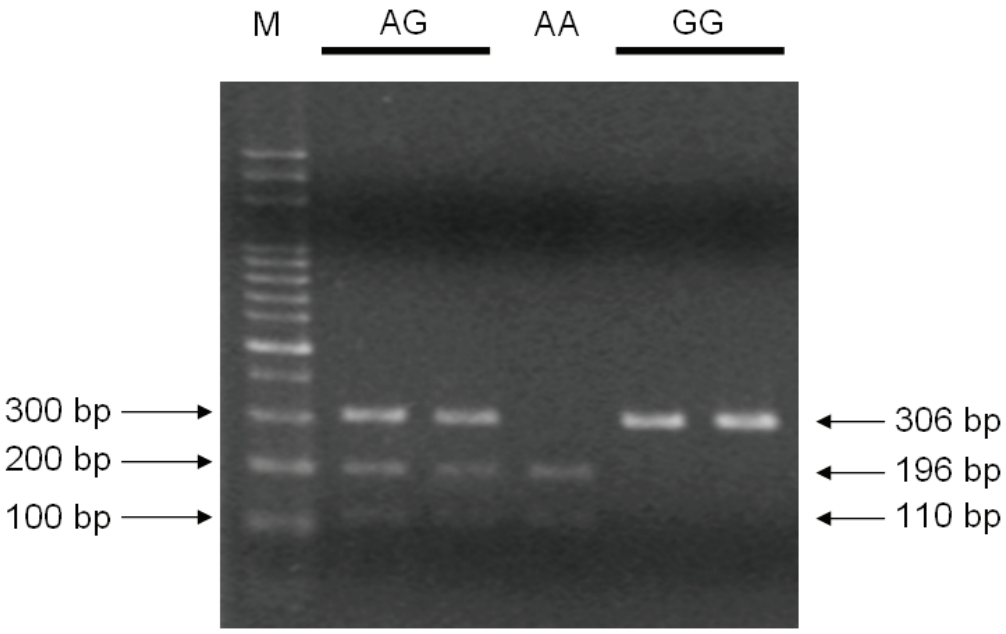

Table I. The characteristics of gastric cancer patients and controls.

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.

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

Genotype	Controls	%	Patients	%	P^a
A-1419G rs3754093					0.5857
AA	75	41.9%	68	38.0%	
AG	82	45.8%	83	46.4%	
GG	22	12.3%	28	15.6%	
C-908G 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

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

Allele	Controls	%	Patients	%	P^a
A-1419G rs3754093					0.3143
Allele A	232	64.8%	219	61.2%	
Allele G	126	35.2%	139	38.8%	
C-908G 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.

Table IV. *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	35		1.00
Patients	68	60		**2.07 (1.22-3.50)**[c]
Non-smokers			0.9337	
Controls	43	19		1.00
Patients	35	16		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.