Association of Genetic Polymorphisms of *EXO1* Gene with Risk of Breast Cancer in Taiwan

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Abstract. *The aim of the present study was to evaluate the association between the polymorphisms of the EXO1 gene and the risk of breast cancer in central Taiwan. Patients and Methods: In this hospital-based study, the association of EXO1 A1419G (rs3754093), C908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P (rs9350) and C3114T (rs851797) polymorphisms with breast cancer risk in a central Taiwanese population was investigated. In total, 1,272 patients with breast cancer and 1,272 age- and gender-matched healthy controls recruited from the China Medical University Hospital were genotyped. Results: A significantly different distribution was found in the frequency of the EXO1 K589E genotype, but not the other genotypes, between the breast cancer and control groups. The A allele EXO1 K589E conferred a significantly (p=0.000025) increased risk of breast cancer. As for the rest of the polymorphisms, there was no difference in distribution between the breast cancer and control groups. Conclusion: Our results provide evidence that the A allele of EXO1 K589E may be associated with the development of breast cancer and may be a useful biomarker for breast cancer detection and primary prevention.*

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Breast cancer is the most common cancer in women. Incidence rates of the disease vary considerably by world region, with the highest rates seen in North America (99.4 per 100,000 women) and Europe (62.3 per 100,000 women) (1). Epidemiological studies suggest that the etiology of breast cancer is multifactorial, including exposure to ionizing radiation, high-fat dietary intake, alcohol consumption, and use of hormones or oral contraceptives. However, only a small proportion of women exposed to these external factors developed breast cancer (2, 3), suggesting that genetic susceptibility plays a role in individual risk of breast cancer. Breast cancer seems to be the result of cumulative alterations of oncogenes and tumor suppressor genes in the human genome that lead to clonal growth of progressively malignant cells (3, 4). DNA damage and genome instability are thought to comprise the first step of carcinogenesis. As the DNA repair systems are responsible for removing various types of DNA damage and maintaining genome stability, their functional defects are also very important in the progress of breast cancer (5-7).

The mismatch repair (MMR) system is one of the major DNA repair pathways in human cells and maintains genomic stability, modulates DNA recombination and mediates cell cycle arrest (8). MMR is closed related to the progress of malignancies, and many reports indicated that deficient mutations of the MMR system lead to various types of cancer (9-11). The gene *exonuclease 1* (*EXO1*; MIM #606063) belongs to the MMR system, and also belongs to the RAD2 nuclease family. It is located at chromosome 1q42-q43, contains one untranslated exon followed by 13 coding exons and encodes an 846 amino acid protein (12-14). EXO1 can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells, and with MSH3 in human cells (14-19). Recent findings indicated that mammalian EXO1 is responsible for mutation prevention

Table I. *The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for EXO1 gene polymorphisms.*

Polymorphism (location)	Primers sequences (5'->3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
A1419G	F: AACTGACAGGCACACTTAAG	EcoP15 I	A	386
	R: GTAGAGAAGCCTTCTTACAC		G	144 + 242
C908G	F: GTTAGGTCTACCATAGCCTT	HpyCH4 IV	G	470
	R: TTCATGGTCACTTGTGGCTA		C	225 + 245
A238G	F: AGTCTCTTACCTCTCAGATG	Dpn II	G	367
	R: TACATGCAATCTCTCCACCT		A	178 + 189
C498T	F: AGCGTAGTAAGAATGGCTGA	Stu I	T	323
	R: GATAAGAGAGCAGACGATTC		C	150 + 173
K589E	F: GACACAGATGTAGCACGTAA	Mse I	G	306
	R: CTGCGACACATCAGACATAT		A	110 + 196
G670E	F: AATATGTCTGATGTGTCGCA	Ear I	G	273
	R: CTGCGACACATCAGACATAT		A	71 + 202
C723R	F: ACACCTACAGTCAAGCATAA	HpyCH4 IV	A	264
	R: ACTCTAGGAATCTGATTGCA		G	66 + 198
L757P	F: ATATAAGTCCAATTCTGCAG	Mnl I	T	255
	R: AAGAAACAAGGCAACTCACT		C	102 + 153
C3114T	F: CTACTTGACAACATTACAGA	Mse I	C	602
	R: GAGAACCTGATTGTGTTATA		T	173 + 429

*F and R indicate forward and reverse primers, respectively.

and mice with EXO1 inactivation have reduced survival time and increased risk for tumor development, specifically for lymphoma (20).

In the literature, single nucleotide polymorphisms (SNPs) of DNA repair genes have been associated with susceptibility to several types of cancer, including oral, gastric, prostate, colorectal, lung and breast cancer (21-28). These reports indicated that SNPs of the DNA repair system may affect the genes' functions or expression levels, and the capacity of those gene-related systems will also be affected. Therefore, cancer susceptibility will be higher in people who carry risky genotypes. There are already several SNPs of *EXO1* which have been reported as genetic risk factors of cancer. In 2005, a study investigating a Japanese population found that two polymorphisms of the *EXO1* gene, T439M and P757L, are associated with colorectal cancer risk (26). In 2008, the association between SNPs of *EXO1* and lung cancer susceptibility was examined in a Chinese population, indicating the K589E is associated with lung cancer risk (27). In this study, we have chosen nine SNPs of *EXO1* and investigated their frequency distributions and associations with breast cancer in Taiwan.

Patients and Methods

Study population and sample collection. About one thousand and three hundred breast cancer patients diagnosed with breast cancer by Dr. Wang were recruited at the outpatient clinics of general surgery between 1999-2009 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-cancer healthy volunteers as controls were selected by matching for age, gender and some habits 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 other or unknown origin, and any known familial or genetic diseases. Both groups completed a short questionnaire which included individual habits. 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 leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (21-25, 28). The PCR cycling conditions were: 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. Pairs of PCR primer sequences and restriction enzyme for each DNA product are all listed in Table I.

Statistical analyses. Only those matches with all SNP data (case/control=1272/1272) 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 controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's 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*-value was less than 0.05.

Table II. *Distribution of EXO1 genotypes among breast cancer patients and controls.*

Genotype	Controls	%	Patients	%	P^a
A1419G rs3754093					0.4532
AA	535	42.0%	504	39.6%	
AG	577	45.4%	599	47.1%	
GG	160	12.6%	169	13.3%	
C908G rs10802996					0.6339
CC	728	57.2%	735	57.8%	
CG	426	33.5%	408	32.1%	
GG	118	9.3%	129	10.1%	
A238G rs1776177					0.6242
AA	589	46.3%	581	45.7%	
AG	592	46.5%	587	46.1%	
GG	91	7.2%	104	8.2%	
C498T rs1635517					0.5041
CC	54	4.2%	52	4.1%	
CT	421	33.1%	449	35.3%	
TT	797	62.7%	771	60.6%	
K589E rs1047840					2.5×10^{-5}
AA	33	2.6%	57	4.5%	
AG	341	26.8%	421	33.1%	
GG	898	70.6%	794	62.4%	
G670E rs1776148					0.7293
AA	59	4.6%	64	5.0%	
AG	255	20.1%	267	21.0%	
GG	958	75.3%	941	74.0%	
C723R rs1635498					0.9066
AA	972	76.4%	968	76.1%	
AG	283	22.3%	289	22.7%	
GG	17	1.3%	15	1.2%	
L757P rs9350					0.3503
CC	402	31.6%	433	34.0%	
CT	596	46.9%	563	44.3%	
TT	274	21.5%	276	21.7%	
C3114T rs851797					0.9260
CC	258	20.3%	266	20.9%	
CT	630	49.5%	625	49.1%	
TT	384	30.2%	381	30.0%	

[a]*P*-value based on χ^2 test.

Table III. *Distribution of EXO1 alleles among breast cancer patients and controls.*

Allele	Controls	%	Patients	%	P^a
A1419G rs3754093					0.2428
Allele A	1647	64.7%	1607	63.2%	
Allele G	897	35.3%	937	36.8%	
C908G rs10802996					0.8984
Allele C	1882	74.0%	1878	73.8%	
Allele G	662	26.0%	666	26.2%	
A238G rs1776177					0.5238
Allele A	1770	69.6%	1749	68.8%	
Allele G	774	30.4%	795	31.2%	
C498T rs1635517					0.4109
Allele C	529	20.8%	553	21.7%	
Allele T	2015	79.2%	1991	78.3%	
K589E rs1047840					3.84×10^{-6}
Allele A	407	16.0%	535	21.0%	
Allele G	2137	84.0%	2009	79.0%	
G670E rs1776148					0.3889
Allele A	373	14.7%	395	15.5%	
Allele G	2171	85.3%	2149	84.5%	
C723R rs1635498					0.9324
Allele A	2227	87.5%	2225	87.5%	
Allele G	317	12.5%	319	12.5%	
L757P rs9350					0.4132
Allele C	1400	55.0%	1429	56.2%	
Allele T	1144	45.0%	1115	43.8%	
C3114T rs851797					0.7567
Allele C	1146	45.1%	1157	45.5%	
Allele T	1398	54.9%	1387	54.5%	

[a]*P* based on χ^2 test.

Results

The frequency of the genotypes for the *EXO1* A1419G, C908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T between controls and breast cancer patients are shown in Table II. The genotype distribution of various genetic polymorphisms of *EXO1* K589E was significantly different between breast cancer and control groups ($p<0.05$), while those for all the other polymorphisms were not significant ($p>0.05$) (Table II). To sum up, the AA genotype of *EXO1* K589E was associated with higher susceptibility for breast cancer. Representative PCR-based restriction analyses for the *EXO1* K589E polymorphism are shown in Figure 1.

The frequency of the alleles for the *EXO1* A1419G, *EXO1* C908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T between controls and breast cancer patients is shown in Table III. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between controls and breast cancer patients. Allele frequency distribution of the *EXO1* K589E *A was associated with higher susceptibility for breast cancer (Table III).

Discussion

In order to find potential biomarkers of breast cancer, in this study, we selected nine SNPs of the *EXO1* gene and investigated their associations with the susceptibility for breast 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 breast cancer (Tables II and III).

Among the DNA repair systems, one of the major roles is played by the MMR system. The MMR system is responsible for correcting the mismatch between bases and small insertion/deletion loops. Thus, it is essential in

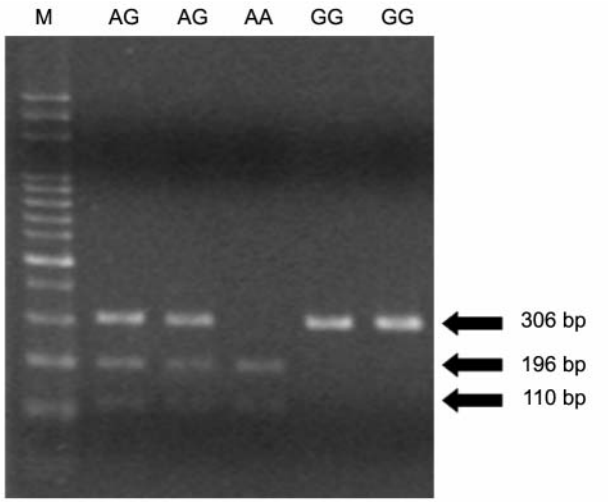

Figure 1. *PCR-based restriction analysis of the EXO1 K589E rs1047840 polymorphism shown on 2.5% agarose electrophoresis. M: 100 bp DNA size marker, G/G: enzyme indigestible homozygote, A/G: heterozygote, and A/A: enzyme digestible homozygote.*

maintaining the integrity of the genome (29, 30). EXO1 is the only exonuclease involved in the human MMR system, playing a critical role as both a 5'-3' and a 3'-5' nuclease and contributing to the overall integrity of the MMR complex (31). Because EXO1 plays a distinctive role in the MMR system, the *EXO1* gene has become a significant target gene and has been widely investigated for its association with risks of various malignancies (32-34).

In this study, we found that *EXO1* K589E was associated with breast cancer susceptibility in Taiwan. The polymorphism is located on exon 12 of the *EXO1* gene and its change causes the 589th amino acid of the Exo1 protein product to be altered from lysine to glutamic acid. The amino acid change at codon 589 might influence the products of *EXO1* mRNA, for K589E was found to be located at an exonic splicing enhancer (ESE) region (27). We propose that the A allele of K589E may affect EXO1 activity, slightly influencing its normal function. As those people with A allele(s) become older, the alterations caused by towards carcinogens may accumulate *via* an increasing of unremoved DNA adducts. Therefore, in individuals who have a risky genetic variant, such as the A allele of K589E, and who are exposed to more cancer- risk modifying factors (such as a smoking habit), the joint effect of genetic and environmental factors will likely synergistically increase their breast cancer susceptibility.

To sum up, to our knowledge this is the first study which has focused on the SNPs of *EXO1* and breast cancer which shows the presence of the A allele of K589E was associated with a higher risk of breast cancer in Taiwan. It is our future work to integrate genomic findings with clinical data to investigate the gene–gene and gene–environment interactions in breast carcinogenesis.

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