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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Key Words: EXO1, polymorphism, breast cancer, carcinogenesis.
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.

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

<table>
<thead>
<tr>
<th>Polymorphism (location)</th>
<th>Primers sequences (5’-&gt;3’)</th>
<th>Restriction enzyme</th>
<th>SNP sequence</th>
<th>DNA fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1419G</td>
<td>F: AACTGACAGGCACACTTTAAG</td>
<td>EcoP15 I</td>
<td>A</td>
<td>386</td>
</tr>
<tr>
<td></td>
<td>R: GTAGAAGACCTTCTTTACAC</td>
<td></td>
<td>G</td>
<td>144 + 242</td>
</tr>
<tr>
<td>C908G</td>
<td>F: GTTAGGTCACCTAAAGGTCTTT</td>
<td>HpyCH4 IV</td>
<td>G</td>
<td>225 + 245</td>
</tr>
<tr>
<td></td>
<td>R: TCTATGGTCACCTTTGCTGTA</td>
<td></td>
<td>C</td>
<td>367</td>
</tr>
<tr>
<td>A238G</td>
<td>F: AGTCTTCTACCTCCTCTAGT</td>
<td>Dpn II</td>
<td>A</td>
<td>178 + 189</td>
</tr>
<tr>
<td></td>
<td>R: TATGGAATCTCTTCCACCT</td>
<td></td>
<td>T</td>
<td>323</td>
</tr>
<tr>
<td>C498T</td>
<td>F: AGCGTAGTAAGATGTTGCTGA</td>
<td>Stu I</td>
<td>C</td>
<td>150 + 173</td>
</tr>
<tr>
<td></td>
<td>R: GTAGAAGAGCAGAGATATTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K589E</td>
<td>F: GACACAGAGTAGAGAGCTAAG</td>
<td>Mse I</td>
<td>A</td>
<td>110 + 196</td>
</tr>
<tr>
<td></td>
<td>R: CTGGGACACATCGAGATAT</td>
<td></td>
<td>G</td>
<td>273</td>
</tr>
<tr>
<td>G670E</td>
<td>F: AAATGTCTGTAGTGGCTCGCA</td>
<td>Ear I</td>
<td>A</td>
<td>71 + 202</td>
</tr>
<tr>
<td></td>
<td>R: CTGGGACACATCGAGATAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C723R</td>
<td>F: ACACCTACAGTAGCAAGCATA</td>
<td>HpyCH4 IV</td>
<td>A</td>
<td>264</td>
</tr>
<tr>
<td></td>
<td>R: ACTCTAGGAACTGTTGCA</td>
<td></td>
<td>G</td>
<td>66 + 198</td>
</tr>
<tr>
<td>L757P</td>
<td>F: ATATAAGTCCATTCCTGCA</td>
<td>Mnl I</td>
<td>T</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>R: AAGAAAGACAGCAGATCTCAG</td>
<td></td>
<td>C</td>
<td>102 + 153</td>
</tr>
<tr>
<td>C3114T</td>
<td>F: CTACTTGACAGACATTAGAGA</td>
<td>Mse I</td>
<td>C</td>
<td>602</td>
</tr>
<tr>
<td></td>
<td>R: GAGACACTGATGGTTGTTATA</td>
<td></td>
<td>T</td>
<td>173 + 429</td>
</tr>
</tbody>
</table>

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

### 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.

### 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...
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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References


