Abstract. Aim: Several epidemiological studies have investigated the association between ataxia telangiectasia mutated (ATM) gene polymorphisms and breast cancer risk. However, published data are still inconclusive and there are no such studies for Taiwan. Thus, the polymorphic variants of ATM were investigated for their association with breast cancer in Taiwan for the first time here. Patients and Methods: In this hospital-based matched case–control study, associations of seven ATM single nucleotide polymorphisms (rs600931, rs652311, rs227060, rs227292, rs624366 and rs189037) with breast cancer risk in a Taiwanese population were investigated. One thousand two hundred and thirty-two patients with breast cancer and the same number of age-matched healthy controls recruited were genotyped and analyzed. Results: There was a slight difference between breast cancer and control groups in the distributions of their genotypic (p=0.0774) and allelic frequencies (p=0.0217) in the rs189037 polymorphism. As for the other six polymorphisms there was no differential distribution. Conclusion: Our data indicate that ATM polymorphism is associated with breast cancer, and the A allele of ATM rs189037 is a minor risky biomarker of breast cancer in Taiwan. The gene–gene and gene–environment interactions of ATM with other factors is worthy of further investigation.

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Therefore, in this study, we aimed at revealing the genotypic frequencies of seven polymorphisms of the ATM gene at rs600931, rs652311, rs227060, rs227092, rs624366, rs189037 and rs228589, and investigating the association of ATM genotypes with breast cancer susceptibility in Taiwanese females.

Patients and Methods

Study population and sample collection. One thousand two hundred and thirty-two cancer patients diagnosed with breast 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 including histological details were all defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The same number of age-matched non-breast cancer healthy volunteers as controls were selected 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 familial or genetic diseases. Both groups completed a short questionnaire which included 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.

Single nucleotide polymorphism (SNP) selection and genotyping conditions. Five tagging polymorphisms were selected with $r^2 > 0.8$ and minor allele frequency $>5\%$ in the Chinese population from the HapMap project including rs600931, rs624366, rs228589, rs227092, rs227060 (18). Because the variants in the 5‘ and 3‘ untranslated regions of the ATM gene may also play roles in modifying its functions, two SNPs (rs189037 and rs652311) with minor allele frequencies $>5\%$ were also selected for investigation. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous reports (19-28). The primer sequences and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) conditions for ATM polymorphisms are summarized in Table I.

Statistical analyses. Only those matches with all SNP data (case/control=1232/1232) 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 ATM SNPs in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s chi-square test was used to compare the distribution of the ATM genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data were recognized as significant when the statistical $p$-value was less than 0.05.

Results

The clinical characteristics and analysis of the recruited 1232 female breast cancer patients and 1232 age-matched female controls are shown in Table II. There were no significant differences between both groups in their ages at their enrollment, first baby birth, and menopause. The tumor sites are also recorded (Table II). Among the seven SNPs investigated, the genotypes of
ATM rs189037 were slightly differently distributed between breast cancer and control groups \((p=0.0774)\), while those for rs600931, rs652311, rs624366, rs228589, rs227092 and rs227060 were not significant \((p>0.05)\) (Table III).

The frequencies of the alleles for the seven ATM SNPs between controls and breast cancer patients are shown and compared in Table IV. Allelic frequency distributions of the ATM rs189037 allele A were 41.7% and 38.5% in case and control groups, respectively. The allelic distribution of ATM rs189037 allele A is significantly different between control and case groups \((p=0.0217)\). To sum up, the A allele at ATM rs189037 seems to be associated with higher susceptibility for breast cancer.
Discussion

The \textit{ATM} gene has been reported to play a role in DNA damage-repair pathways and cell-cycle controlling checkpoints, which are ultimately involved in cancer susceptibility (9, 29, 30). Although some studies have reported that female \textit{ATM} heterozygous carriers have an increased risk of breast cancer in Western countries (16, 17, 31-33), there is no evidence regarding the role of \textit{ATM} as a genetic marker for breast cancer in Taiwan, where the breast cancer is unique in high prevalence, high mortality, and early onset.

Therefore, the main purpose of the present study was to investigate the association between \textit{ATM} polymorphisms and breast cancer risk in Taiwan. All the seven polymorphisms of \textit{ATM} are located in non-coding regions, and might influence the splicing process and RNA stability such as for IVS10-6 T>G, in which variant G was shown to lead to incorrect splicing of exon 11 and exon skipping, resulting in a frameshift and subsequent truncation of the protein at amino acid 419 residue (34). In this study, the A allele of \textit{ATM} rs189037 polymorphism was associated with Taiwan breast cancer (Tables III and IV), while the other six polymorphisms investigated were not. Although the \textit{ATM} rs189037 genetic variation does not direct result in an amino acid coding change, it is reasonable to suspect alternative splicing, intervention, modification, determination or involvement of this SNP influences the expression level or stability of the \textit{ATM} protein.

Our sample size is very large, and our data are directly described without any adjustment. We have also compared all the frequencies of \textit{ATM} polymorphism variant alleles with those in other Asian population, for example, the minor A allele frequency of \textit{ATM} rs189037 was 38.5% in our control group and 38.9-50.0% for the Asian population as given in the NCBI website, and our sample size (1232) is more than thirteen-fold theirs. The distributions of \textit{ATM} at the seven loci were in Hardy-Weinberg equilibrium, which suggest that there was little selection bias for participant enrolment in terms of genotypes existed in this study. Therefore, the need for the present results to be verified in further larger studies is urgent. However, the interactions of \textit{ATM} genotypes with other factors, such as estrogen exposure, may be further investigated. The interaction of \textit{ATM} with other genes, such as \textit{CHK2}, \textit{MDM2}, \textit{NBS1} and \textit{BRCA1}, is also of interest.

In conclusion, this is the first report to investigate the association between \textit{ATM} gene polymorphisms and breast cancer in Taiwan. Our findings suggested that \textit{ATM} rs189037 was associated with breast cancer susceptibility. The \textit{ATM} rs189037 A allele might become a potential biomarker for the breast cancer prediction.

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