Association between Ataxia Telangiectasia Mutated Gene Polymorphisms and Breast Cancer in Taiwanese Females

HWEI-CHUNG WANG[1,*], WEN-SHIN CHANG[1,*], RU-YIN TSAI[1,*], CHIA-WEN TSAI[1,2], LIANG-CHIH LIU[1], CHEN-HSIEN SU[1], HUI-NI CHENG[1], YUNG-AN TSOU[1], SHUNG-SHUNG SUN[1], CHENG-CHIEH LIN[1,3] and DA-TIAN BAU[1,2,4]

[1]Terry Fox Cancer Research Laboratory and [3]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan, R.O.C.; [2]Graduate Institutes of Basic Medical Science and [4]Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.

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.*

*These Authors contributed equally to this work.

Correspondence to: Da-Tian Bau, Ph.D., Terry Fox Cancer Research Lab, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan. Tel: +886 422053366 Ext. 3312, Fax: +886 422053366, e-mail: datian@mail.cmuh.org.tw; artbau1@yahoo.com.tw

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Breast cancer is the most common malignancy in females worldwide. Except for the high breast cancer risk in *breast cancer gene 1* (*BRCA1*) and *BRCA2* mutation carriers, as well as the risk for breast cancer in certain rare syndromes caused by mutations in *tumor protein 53 (TP53), serine/threonine kinase 11 (STK11), phosphatase and tensin homologue deleted on chromosome ten (PTEN), cadherin-1 (CDH1), neurofibromatosis type I (NF1)* and *Nijmegen breakage syndrome 1 (NBS1)*, familial clustering of breast cancer remains largely unexplained (1-4). Ataxia telangiectasia is an autosomal recessive disorder that affects many parts of the body and leads to increased risk of malignancy, including breast cancer (5-7), and is caused by mutations in the ataxia telangiectasia-mutated (*ATM*) gene, a member of the phosphoinositide-3-kinase family (8, 9).

Carcinogens may induce various types of DNA damage, including DNA adducts, and single- and double-strand breaks (DSBs). Among the different types of DNA damage and their associated DNA repair proteins, ATM plays a critical role in the recognition, signaling, and repairing of DNA DSBs (8). In response to DSB induction, ATM is rapidly activated and can phosphorylate various downstream substrates, some of which are key factors in the regulation of cell cycle arrest, DNA repair, and apoptosis. For example, ATM is an upstream factor of tumor-suppressor protein TP53 and regulates progression of the cell cycle and apoptosis by activation and stabilization of p53 (10, 11). ATM can also interact with and phosphorylate oncogenic protein (murine double minute 2; MDM2) (12), checkpoint kinase (cell-cycle-checkpoint kinase 2; CHK2) (13), tumor-suppressor protein BRCA1 (14), and DNA-repair protein NBS1 (15). Moreover, recent large epidemiological and molecular analyses of ATM indicate that *ATM* mutations are low-penetrance susceptibility alleles of breast cancer (16, 17) However, there is no literature investigating the *ATM* polymorphisms that may directly contribute to susceptibility of breast cancer in Taiwan.

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

Reference sequencing #	Function variation	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
rs600931	Intron	F: 5'-CTGGCCTAAGAGAAAAATATTGC-3'	HpyCH4V	G	100 bp
		R: 5'-AATGTGTCTTGGGAAAGATGAC-3'		A	78 + 22 bp
rs189037	5'UTR	F: 5'-GCTGCTTGGCGTTGCTTC-3'	MscI	G	287 bp
		R: 5'-CATGAGATTGGCGGTCTGG-3'		A	176 + 111 bp
rs652311	3'UTR	F: 5'-GTAGTGTTTCTTAGTCGCCTCCTGTC-3'	Taqa	A	133 bp
		R: 5'-ACCAGGATCTTTGCACTTGTCAT-3'		G	108 + 25 bp
rs624366	Intron	F: 5'-TTTATTTTGCTAACTTTAACTCTGTA-3'	RasI	G	119 bp
		R: 5'-TGTTCAACAAATATGAGATGC-3'		C	94 + 25 bp
rs228589	Promotor	F: 5'-TGTGGTTCCTGCTGTGGTTT-3'	FokI	A	195 bp
		R: 5'-CCGCCAGTCTCAACTCGTAA-3'		T	104 + 91 bp
rs227092	3'UTR	F: 5'- AGTATGGTGAAACCCTGTC-3'	HpyCH4IV	T	481 bp
		R: 5'- AAGAAGCCCAATGGATAG-3'		G	265 + 216 bp
rs227060	Intron	F: 5'- AGCCCTAAAATACTCAAAAGCTTCAC-3'	BfuAI	T	128 bp
		R: 5'- AGCACACGGAAACTCTCCTTCT-3'		C	94 + 34 bp

F and R indicate forward and reverse primers, respectively.

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, and 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).

The frequencies of the *ATM* genotypes between controls and breast cancer patients are shown and compared in Table III. Among the seven SNPs investigated, the genotypes of

Table II. *Distribution of demographic data of breast cancer patients and the matched controls.*

Characteristic		Controls (n=1232)			Patients (n=1232)			*P*-value
		n	%	Mean (SD)	n	%	Mean (SD)	
Age at enrollment (years)	<40	359	29.1%		362	29.4%		0.89[a]
	40-55	558	45.3%		547	44.4%		
	>55	315	25.6%		323	26.2%		
Age at menarche (years)				13.7 (0.7)			13.6 (0.6)	0.79[b]
Age at first baby birth (years)				28.4 (1.2)			28.8 (1.4)	0.63[b]
Age at menopause (years)				48.8 (1.8)			49.3 (2.0)	0.59[b]
Site	Unilateral				1198	97.2%		
	Bilateral				34	2.8%		

Statistical results based on [a]Chi-square or [b]unpaired Student's *t*-test.

Table III. *Distribution of ATM genotypes among breast cancer patients and controls.*

Genotype	Controls		Patients		*P*-value[a]
	n	%	n	%	
rs600931					0.8668
GG	478	38.8%	468	38.0%	
AG	518	42.0%	531	43.1%	
AA	236	19.2%	233	18.9%	
rs189037					0.0774
GG	474	38.5%	428	34.7%	
AG	567	46.0%	580	47.1%	
AA	191	15.5%	224	18.2%	
rs652311					0.7687
GG	493	40.0%	508	41.2%	
AG	635	51.5%	617	50.1%	
AA	104	8.5%	107	8.7%	
rs624366					0.4948
GG	514	41.7%	538	43.7%	
CG	600	48.7%	589	47.8%	
CC	118	9.6%	105	8.5%	
rs228589					0.4088
TT	436	35.4%	468	38.0%	
AT	547	44.4%	525	42.6%	
AA	249	20.2%	239	19.4%	
rs227092					0.6972
GG	479	38.9%	497	40.3%	
GT	515	41.8%	510	41.4%	
TT	238	19.3%	225	18.3%	
rs227060					0.7645
CC	552	44.8%	537	43.6%	
CT	522	42.4%	527	42.8%	
TT	158	12.8%	168	13.6%	

[a]Based on Chi-square test.

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).

Table IV. *Distribution of ATM alleles among breast cancer patients and controls.*

Allele	Controls		Patients		*P*-value[a]
	n	%	n	%	
rs600931					0.8389
Allele G	1474	59.8%	1467	59.5%	
Allele A	990	40.2%	997	40.5%	
rs189037					0.0217
Allele G	1515	61.5%	1436	58.3%	
Allele A	949	38.5%	1028	41.7%	
rs652311					0.7181
Allele G	1621	65.8%	1633	66.3%	
Allele A	843	34.2%	831	33.7%	
rs624366					0.2630
Allele G	1628	66.1%	1665	67.6%	
Allele C	836	33.9%	799	32.4%	
rs228589					0.2247
Allele A	1419	57.6%	1461	59.3%	
Allele T	1045	42.4%	1003	40.7%	
rs227092					0.3665
Allele G	1473	59.8%	1504	61.0%	
Allele T	991	40.2%	960	39.0%	
rs227060					0.4538
Allele C	1626	66.0%	1601	65.0%	
Allele T	838	34.0%	863	35.0%	

[a]Based on Chi-square test.

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 *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 *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 *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 *ATM* polymorphisms and breast cancer risk in Taiwan. All the seven polymorphisms of *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 *ATM* rs189037 polymorphism was associated with Taiwan breast cancer (Tables III and IV), while the other six polymorphisms investigated were not. Although the *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 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 *ATM* polymorphism variant alleles with those in other Asian population, for example, the minor A allele frequency of *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 *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 *ATM* genotypes with other factors, such as estrogen exposure, may be further investigated. The interaction of *ATM* with other genes, such as *CHK2, MDM2, NBS1* and *BRCA1*, is also of interest.

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

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