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Association Between DNA Repair Gene ATM Polymorphisms and Oral Cancer Susceptibility

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Objectives/Hypothesis: The ataxia-telangiectasia mutated (ATM) is thought to play a major role in the caretaking of the overall genome stability, and its mutations have been implicated in human cancers. However, the role of ATM polymorphisms in oral carcinogenesis is largely unexplored. Thus, the polymorphic variants of ATM were first investigated for their association with oral cancer susceptibility.

Study Design: In this hospital-based, case-control study, associations of seven ATM single nucleotide polymorphisms with oral cancer risk in a Taiwanese population were investigated.

Methods: A total of 620 patients with oral cancer and 620 healthy controls were genotyped and analyzed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: There were significant differences between oral cancer and control groups in the distributions of their genotypes ($P = 3.71E\text{-}6$) and allelic frequencies ($P = 5.09E\text{-}6$) in the ATM rs189037 polymorphisms. In the other six polymorphisms, there was no differential distribution between both groups. There are synergistic joint interactions of ATM genotyping with smoking, alcohol drinking, and betel quid chewing.

Conclusions: The ATM rs189037 A allele is correlated with oral cancer susceptibility, and this polymorphism may be a useful marker for oral cancer prevention and early detection.

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INTRODUCTION

Oral cancer, which is a commonly diagnosed cancer all over the world,[1,2] has the highest incidence of all head and neck cancers in Taiwan.[3] Three environmental factors—tobacco smoking, alcohol drinking, and betel quid chewing—are the major causes of oral cancer in Taiwan, while the genomic etiology of oral cancer is of great interest but largely unknown. The human DNA repair system takes care of the genome and protects it from various insults caused by endogenous and environmental agents,[4] and mutations or defects in the DNA repairing system are thought to be essential for tumorigenesis.[5,6] Therefore, it is logical to suspect that some genetic variants of DNA repair genes might contribute to oral cancer pathogenesis.

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, ataxia-telangiectasia mutated (ATM) plays a critical role in the recognition, signaling, and repairing of DNA DSBs.[7] ATM is the product that mutated in autosomal recessive disease ataxia-telangiectasia (AT) and a member of the phosphoinositide 3-kinase family.[8,9] In response to DSBs 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 MDM2,[12] checkpoint kinase CHK2,[13] tumor-suppressor protein BRCA1,[14] and DNA-repair protein NBS1.[15] Moreover, recent large epidemiologic and

TABLE I.
The Primer Sequences, Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) Conditions for ATM Gene Polymorphisms.

Polymorphisms (Locations)	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	104 + 91 bp
		R: 5′-CCGCCAGTCTCAACTCGTAA-3′		T	195 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.
ATM = ataxia-telangiectasia mutated; SNP = single-nucleotide polymorphism.

molecular analyses of ATM indicate that ATM mutations are low-penetrance susceptibility alleles of breast[16,17] and lung cancers.[18] Therefore, the polymorphisms of the ATM gene are plausible candidates that may contribute to susceptibility to oral cancer.

In this study, we determined the genotypic frequencies of seven polymorphisms of the ATM gene at rs600931, rs652311, rs227060, rs227292, rs624366, rs189037, and rs228589, and investigated the joint effects of ATM genotypes with environmental factors, smoking, alcohol drinking, and betel quid chewing on oral cancer susceptibility. To the best of our knowledge, this is the first study carried out to evaluate the contribution of ATM polymorphisms in oral oncology all over the world.

MATERIALS AND METHODS

Study Population and Sample Collection

Six hundred twenty patients diagnosed with oral cancer were recruited at the outpatient clinics of general surgery from 1998 to 2009 at the China Medical University Hospital, Taiwan. All patients voluntarily participated, completed a self-administered questionnaire, and provided peripheral blood samples. The questionnaire administered to the subjects included questions on history and frequency of alcohol consumption, betel quid chewing, and smoking habits. Self-reported alcohol consumption, betel quid chewing, and smoking habits were evaluated and classified as categorical variables. Information on these factors was obtained, and usage of more than twice a week for years was classified as "ever." The same amounts of non-oral cancer healthy people as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consents were 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 Chinese population from HapMap project[19] including rs600931, rs624366, rs228589, rs227092, and rs227060. Because the variants in 5′ and 3′ untranslated regions of the ATM gene may also play roles in modifying of its functions, two SNPs (rs189037 and rs652311) with their minor allele frequencies >5% were also selected to investigate. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous papers.[20–29] The primer sequences, polymerase chain reaction and polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for ATM gene polymorphisms are summarized in Table I. For quality control, 10% of the samples were direct sequenced to confirm the PCR-RFLP analyses. T1

Statistical Analyses

Only those matches with all SNPs data (case/control = 620/620) were selected for 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 ATM SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson χ^2 test or Fisher exact test (when the expected number in any cell was < five) was used to compare the distribution of the ATM genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data was recognized as significant when the statistical P value was <.05.

RESULTS

The clinical characteristics and analysis of 620 recruited oral cancer patients and 620 age- and gender-matched controls are shown in Table II. There were no T2

TABLE II.
Characteristics of Oral Cancer Patients and Controls.

	Controls (n = 620)			Patients (n = 620)			
Characteristics	n	%	Mean (SD)	n	%	Mean (SD)	*P**
Age (years)			61.3 (8.6)			62.5 (10.4)	.73
Gender							.72
Male	582	93.9		586	94.5		
Female	38	6.1		34	5.5		
Indulgence							
Cigarette smokers	443	71.5		458	73.9		.37
Betel quid chewers	382	61.6		399	64.4		.35
Alcohol drinkers	413	66.6		441	71.1		.10
Histology							
Tongue				303	48.9		
Buccal mucosa				180	29.0		
Mouth floor				36	5.8		
Retromolar trigone				27	4.4		
Alveolar ridge				17	2.7		
Palate				14	2.3		
Lip				13	2.1		
Others				30	4.8		

*P based on χ^2 test.
SD = standard deviation.

significant differences between both groups in their age, sex, and individual habits (Table II).

The frequencies of the ATM genotypes between controls and oral cancer patients are shown and compared in T3 Table III. Among the seven SNPs investigated, the genotypes of ATM rs189037 was differently distributed between oral cancer and control groups ($P = 3.71$*10-6), while those for rs600931, rs652311, rs624366, rs228589, rs227092, and rs227060 were not significant ($P > .05$) (Table III).

The frequencies of the alleles for the seven ATM SNPs between controls and oral cancer patients are T4 shown and compared in Table IV. Allele frequency distributions of the ATM rs189037 allele A are 48.5% 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 ($P =$ 5.09*10-6), and ATM rs189037 allele A seems to be associated with higher susceptibility for oral cancer.

Considering potential gene-environment interactions between ATM gene and oral cancer-related individual habits, the risk of oral cancer related to ATM genotypes was further examined with stratification by smoking, alcohol drinking, and betel quid chewing status T5 (Table V). Compared with G/G plus G/A genotype, the A/A variant genotype significantly enhanced the risk in the smokers, alcohol drinkers, and betel quid chewers with the odds ratios of 1.95, 1.61, and 2.05, respectively (Table V).

DISCUSSION

Mutations in the ATM gene, which cause insufficient DNA damage surveillance, allow damaged cells to proceed into mitosis, which eventually results in increased cancer susceptibility.[30] In recent years, investigations have shown abnormal expression of ATM protein in oral cancer cells, and overexpression of ATM may be one of the early events in the oral carcinogenesis.[21] However, the genetic polymorphisms associated with oral cancer are not found yet.[31] The significant influence of ATM polymorphisms on micronuclei formation is biologically plausible because ATM participates in DSB repairing. In human, the initial step in homologous recombination (HR) and the nonhomologous end-joining (NHEJ) is the recognition and signaling of DNA DSBs by the NBS1-MRE11-RAD50 protein complex.[32] In HR, repair involves a strand exchange reaction catalyzed by Rad51 and facilitated by Rad52 through direct interaction. BRCA2 interacts directly with Rad51 and indirectly with BRCA1.[33] ATM regulates BRCA1 and NBS1 by phosphorylation.[34–36] Molecular study showed that ATM mutant cells behaved at a significantly increased rate of DSBs induced by ionizing radiation.[37]

Several previous case-control studies indicated that haplotype of ATM was associated with breast[16,17,38,39] and lung cancers.[18,40,41] This is the first study to investigate the association between ATM gene polymorphisms and oral cancer risk. All seven polymorphisms of ATM are located in the noncoding region, and might influence the splicing process and RNA stability as the IVS10-6 T>G. The IVS10-6G mutation 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.[42,43] Our study revealed that the ATM rs189037 polymorphism was associated with the susceptibility to oral cancer (Tables III, IV), but the

TABLE III.
Distribution of ATM Genotypes Among Oral Cancer Patients and Controls.

Genotype	Number of Controls	%	Number of Patients	%	*P**
rs600931					.8192
GG	230	37.1	237	38.2	
AG	271	43.7	260	41.9	
AA	119	19.2	123	19.9	
rs189037					
GG	239	38.5	181	29.2	3.71*10-6
AG	285	46.0	277	44.7	
AA	96	15.5	162	26.1	
rs652311					
GG	242	39.0	250	40.3	.6339
AG	324	52.3	309	49.8	
AA	54	8.7	61	26.1	
rs624366					
GG	266	42.9	291	46.9	.3545
CG	301	48.5	278	44.9	
CC	53	8.6	51	8.2	
rs228589					
TT	219	35.3	238	38.4	.5349
AT	269	43.4	256	41.3	
AA	132	21.3	126	20.3	
rs227092					
GG	240	38.7	251	40.5	.5457
GT	252	40.6	256	41.3	
TT	128	20.7	113	18.2	
rs227060					
CC	279	45	266	42.9	.4518
CT	260	41.9	258	41.6	
TT	81	13.1	96	15.5	

*P based on χ^2 test.
ATM = ataxia-telangiectasia mutated.

TABLE IV.
Distribution of ATM Alleles Among Oral Cancer Patients and Controls.

Allele	Number of Controls	%	Number of Patients	%	*P**
rs600931					.9025
Allele G	731	59.0	734	59.2	
Allele A	509	41.0	506	40.8	
rs189037					
Allele G	763	61.5	639	51.5	5.09*10-6
Allele A	477	38.5	601	48.5	
rs652311					
Allele G	808	65.2	809	65.2	.9664
Allele A	432	34.8	431	34.8	
rs624366					
Allele G	833	67.2	860	69.4	.2441
Allele C	407	32.8	380	30.6	
rs228589					
Allele A	707	57.0	732	59.0	.3091
Allele T	533	43.0	508	41.0	
rs227092					
Allele G	732	59.0	758	61.1	.2864
Allele T	508	41.0	482	38.9	
rs227060					
Allele C	818	66.0	790	63.7	.2390
Allele T	422	34.0	450	36.3	

*P based on χ^2 test.
ATM = ataxia-telangiectasia mutated.

other six polymorphisms were not. Although the ATM rs189037 genetic variation does not directly result in amino acid coding change, it is plausible to suspect the alternative splicing, intervention, modification, determination, or involvement of this SNP influences the expression level or stability of the ATM protein. Possibly, there may be LD between ATM rs189037 polymorphism and other SNPs in its exons, resulting in functional polymorphism and predisposing to oral carcinogenesis.

The large enough sample size and concise data analysis without adjustment strengthen the accuracy and reliability of our finding, and the frequencies of ATM polymorphisms variant alleles were similar to those reported in the National Center for Biotechnology Information (NCBI) website in the Asian population

TABLE V.
Odds Ratios (ORs) for ATM rs189037 Genotype and Oral Cancer After Stratified by Individual Behavior Status.

	Number of Controls %		Number of Cases %		OR* (95% CI)	*P* Value
Smokers						
GG+AG	374	84.4	337	73.6	1.000 (ref)	.0001†
AA	69	15.6	121	26.4	1.95 (1.400–2.708)†	
Alcohol drinkers						
GG+AG	318	83.2	301	75.4	1.000 (ref)	.0020†
AA	64	16.8	98	24.6	1.61 (1.137–2.301)†	
Betel quid chewers						
GG+AG	319	83.5	284	71.2	1.000 (ref)	.0001†
AA	63	16.5	115	28.8	2.05 (1.450–2.899)†	

*The ORs were estimated with multivariate logistic regression analysis.
†Statistically identified as significant.
ATM = ataxia-telangiectasia mutated; CI = confidence interval.

studies. For example, minor A allele frequencies of ATM rs189037 are 15.5% in our control group and 15.6% to 18.2% for Asian population in NCBI. The distributions of ATM at the seven loci were in Hardy-Weinberg equilibrium, which suggests no selection bias for the subjects' enrollments in terms of genotypes. Therefore, the need for the present results to be verified in further larger studies is not so urgent.

Three environmental factors—cigarette smoking, alcohol consumption, and betel quid chewing—were reported to be closely related to oral carcinogenesis. Tobacco and alcohol consumption leads to damage to the cells and to the genetic code.[44] If these alterations to DNA structures are left unrepaired, genetic changes can accumulate, which may result in cell-cycle dysregulation, autonomous growth, and development of invasive mechanisms, leading to carcinoma.[45] Smoking is associated with free radical-induced DNA damage and strand breaks,[46] and tobacco smoke contains some potential carcinogens including polycyclic aromatic hydrocarbons, aromatic amines, tobacco nitroamines, and BPDE, which form DNA bulky adducts and DNA strand breaks.[47] Previously, our group provided evidence for the interactions between XRCC4 and these environmental factors.[22] It was found that there were positive joint effects among smokers and betel quid chewers, but not alcohol drinkers, with variant XRCC4 intron3 genotypes. These findings suggested that genetic variants in the DSB repair system may enhance the genomic vulnerability to smoking- and betel quid chewing-caused DNA insults, leading to oral carcinogenesis. Betel quid chewing can increase the risk of oral cancer significantly, and it is a well-known fact in Taiwan. In this study, we found there are joint effects between ATM genotype and each of the three habits (Table V). The possible carcinogenesis process is that all of these habits can cause a lot of free radicals in our oral cavity, which make plenty of DNA injuries in oral mucous. In the recruited "normal" people, those injuries can be removed immediately after their forming. Once the capacity of the DNA repair system is not good enough, these DNA injuries will accumulate in the genome of the cell, and some of these cells may become unregulated tumor. Therefore, people who carry both risky habits and ATM rs189037A genotype will have the higher risk of oral cancer as observed.

CONCLUSIONS

In conclusion, this is the first report to investigate the association between ATM gene polymorphisms and oral cancer. Our findings suggested that ATM rs189037 was associated with oral cancer susceptibility, and it has joint effects with smoking, alcohol drinking, and betel quid chewing on individual oral cancer risk. The ATM rs189037 A allele might become a potential biomarker for the oral oncology prediction, and this study may also provide a valuable insight into the oral carcinogenesis.

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