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ATM polymorphisms and risk of lung cancer among never smokers

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ABSTRACT

The ataxia-telangiectasia mutated (ATM) gene, an important caretaker of overall genome stability, is thought to play a role in the development of human malignancy. Therefore, we hypothesized that sequence variants in *ATM* may influence the disposition to lung cancer. In this hospital-based matched case-control study, nine *ATM* single nucleotide polymorphisms (rs189037, rs228597, rs228592, rs664677, rs609261, rs599558, rs609429, rs227062, and rs664982) were genotyped in 730 lung cancer patients and 730 healthy controls. Pairwise linkage disequilibrium among nine polymorphisms in the *ATM* gene was very high. None of the main effects of any of the *ATM* polymorphisms were related to the risk of lung cancer. Interestingly, *ATM* polymorphisms were significantly associated with lung cancer among never smokers, and the association was modulated by low-level exposure to carcinogens such as environmental tobacco smoke. When the haplotypes of nine *ATM* polymorphism sites were studied, no overall association between *ATM* haplotypes and risk of lung cancer was found. However, the frequency distribution of haplotypes between lung cancer cases and controls was significant in the never smokers ($P = 0.009$), demonstrating that haplotypes have a significant effect on the risk of lung cancer. In conclusion, we found that never smokers with sequence variants of the *ATM* gene may be at increased risk for lung cancer. Our data also suggest this association may be further modified by exposure to environmental tobacco smoke. This study suggests support to the literature that *ATM* polymorphisms and environmental tobacco smoke exposure have a role in lung carcinogenesis among never smokers.

1. Introduction

Lung cancer is the leading cause of cancer mortality both worldwide and in Taiwan in particular [1]. Although cigarette smoking is the major risk factor in the development of lung cancer, only 10–15% of all smokers develop lung cancer, suggesting that there is great individual variation in susceptibility to lung cancer carcinogens [2–4]. Several genes encoding for DNA repair molecules have been investigated as candidates for lung-cancer-susceptibility genes [5–7].

Among the different types of DNA damage, ataxia-telangiectasia mutated (ATM) plays a key role in the recognition, signaling, and repair of DNA damage, especially DNA double-strand breaks (DSBs) [8]. ATM is the product of the gene that mutated in autosomal recessive disease ataxia-telangiectasia (AT) and a member of the phosphoinositide 3-kinase family [9,10]. 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* [11,12]. ATM can also interact with and phosphorylate oncogenic protein MDM2 [13], checkpoint kinase CHK2 [14], tumor-suppressor protein BRCA1 [15], and DNA-repair

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; DSBs, DNA double-strand breaks; ETS, environmental tobacco smoke; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; OR, odds ratio; MAF, miner allele frequency; SNP, single nucleotide polymorphism.

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protein NBS1 [16]. Moreover, recent large epidemiological and molecular analyses of *ATM* indicate that *ATM* mutations are low-penetrance breast cancer susceptibility alleles conferring about a twofold risk of breast cancer [17,18]. Therefore, common polymorphisms of the *ATM* gene are plausible candidates that may contribute to susceptibility to lung cancer.

In this study, we applied a matched case-control approach to test the hypothesis that sequence variants in *ATM* may influence the disposition to lung cancer. Furthermore, since cigarette smoking may induce DNA damage and a reduced DNA repair capacity is associated with lung cancer [4,19], we hypothesized that the association might be modulated by exposure to cigarette smoking. Therefore, we investigated the gene–environment interaction between the *ATM* genotypes and smoking habits in lung cancer risk. Interestingly, *ATM* polymorphisms were significantly associated with lung cancer among never smokers rather than smokers, and the association was modulated by low-level exposure to carcinogens, such as environmental tobacco smoke (also called ETS, second hand smoking, or passive smoking).

2. Materials and methods

2.1. Study population

The current study recruited 730 lung cancer patients and 730 cancer-free controls from the Genetic Epidemiological Study of Lung Adenocarcinoma (GELAC). Further details about the design of this collaborative project are described in previously published material [20,21]. Briefly, the study case subjects were 730 patients diagnosed with primary lung cancer admitted to six cooperative hospitals in Taiwan, between January 2002 and December 2006. There were no age, gender, tumor histology, or stage restrictions. The 730 control subjects were cancer-free individuals randomly selected from the health examination clinics of the same hospitals during the same time period of case recruitment. The control subjects were frequency matched (1:1) to the cases on the bases of age, gender, and smoking status (never versus ever). At recruitment, a trained research nurse was assigned to obtain informed consent for the collection of a blood sample and to administer a structured questionnaire. The questionnaire collected information about demographic characteristics, lifestyle factors (such as number of cigarettes smoked), medical history, and family history of cancer. For smoking status, a person who was smoking at least once a day and had been doing so for more than 6 months was regarded as a current smoker. A former smoker was defined as someone who had stopped smoking at least 6 months before either the diagnosis in the cases or the date signed on the informed consent form in the controls. The cumulative cigarette dose (pack-years) was calculated by multiplying by smoking duration (in years) by smoking intensity (in cigarette packs). A never smoker was defined as someone whose cumulative lifetime cigarette dose was equal to 0. ETS exposure was defined as having been around someone else's cigarette smoke on a regular basis and was categorized as an indicator variable equal to 1 if the participant reported exposure to ETS at home, at work, or during leisure activities; otherwise, this was marked as 0. Exposed individuals also reported the number of years of exposure.

2.2. Genotyping analysis

Genomic DNA was extracted from blood samples of all subjects by the conventional phenol/chloroform extraction method. Practically, the *ATM* locus showed the extensive linkage disequilibrium (LD) [22,23]. Thus, our strategy was using data from the International HapMap project (http://www.hapmap.org) to select tagSNPs based on haplotype blocks of the *ATM* gene among Han Chinese with the pairwise correlation coefficient (γ_p^2) >0.8 and minor allele frequency (MAF) >10%. On the other hand, we selected SNPs by using information from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) with MAF > 10% distributed evenly throughout the *ATM* genomic locus (146 kb). From the International HapMap project (three tagSNPs) and from the dbSNP database (six SNPs), a total of nine *ATM* SNPs (rs189037, rs228597, rs228592, rs664677, rs609261, rs599558, rs609429, rs227062, and rs664982) were selected for genotyping. All genotyping was done using the MassARRAY (SEQUENOM, Inc., San Diego, CA, USA) system at the National Genotyping Core of the National Research Program for Genomic Medicine (NRPGM) of Taiwan. The PCR primers and extension primers were designed using Spectro-Designer software (SEQUENOM, Inc., San Diego, CA, USA). The results were confirmed by overlapping 10% of the samples in both genotyping assays, and no inconsistent genotypes were found.

2.3. Statistical methods

Demographic variables between cases and controls were compared using Student's *t*-test for continuous variables and the χ^2 test for categorical variables. To ensure that the controls used were representative of healthy controls from the general population and to exclude the possibility of genotyping error, deviation of the genotype frequencies of each SNP in the control subjects from those expected under the Hardy–Weinberg equilibrium (HWE) was assessed using the goodness-of-fit χ^2 test. We compared the distribution of genotypes between case patients and control subjects by using the Monte Carlo approach in which the simulations were performed 100,000 times [24]. The lung cancer risk associated with the genotypes were estimated as an odds ratio (ORs) and 95% confidence intervals (95% CIs) by conditional logistic regression with adjustment for the effect of possible confounders.

The degree of LD between individual SNPs was examined using Lewontin's coefficient D' and LD correlation coefficient γ^2 [25]. On the basis of the observed frequencies of the selected SNPs, we estimated global haplotype frequencies by using the expectation-maximization algorithm [26]. For this analysis, the procedure HAPLOTYPE in SAS/GENETICS [27] was used. Furthermore, to examine the effect of specific haplotypes involved in lung cancer, we chose the most common haplotype as the reference group and estimated haplotype-specific ORs.

To evaluate effect modification by smoking, stratified analyses were conducted for all polymorphisms to compare the association across exposure categories of smoking status (never smokers, ever smokers); and interactions between the polymorphisms and other covariates were assessed by including the cross-product terms as well as the main effect term in logistic regression models.

Exposure to ETS was analyzed in two ways: as a dichotomous variable (presence/absence of the exposure) and as a quantitative variable (duration of exposure from home and workplace combined).

All statistical tests were performed using SAS, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on two-sided probabilities. The Monte Carlo simulations were performed by using SAS/genetics, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

Selected demographic characteristics of study subjects are shown in Table 1. Among the recruited 730 lung cancer patients, 482 (66.03%) were classified as adenocarcinoma, 101 (13.84%) as squamous cell carcinoma, and 147 (20.13%) as other histological types, including small cell carcinoma, large cell carcinoma, and undifferentiated carcinoma. Cases and controls were similar with regard to the matching factors, age and gender. The cumulative cigarette dose (pack-years) in ever smokers, however, was signifi-