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Int J Cancer. Author manuscript; available in PMC 2012 October 22.

Published in final edited form as:
Int J Cancer. 2007 November 15; 121(10): 2254–2259. doi:10.1002/ijc.22918.

ATM sequence variants associate with susceptibility to non-small cell lung cancer

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Abstract

ATM gene mutations have been implicated in many human cancers. However, the role of *ATM* polymorphisms in lung carcinogenesis is largely unexplored. We conducted a case-control analysis of 556 Caucasian non-small-cell lung cancer (NSCLC) patients and 556 controls frequency-matched on age, gender and smoking status. We genotyped 11 single nucleotide polymorphisms of the *ATM* gene and found that compared with the wild-type allele-containing genotypes, the homozygous variant genotypes of *ATM*08 (rs227060) and *ATM*10 (rs170548) were associated with elevated NSCLC risk with ORs of 1.55 (95% CI: 1.02–2.35) and 1.51 (0.99–2.31), respectively. *ATM* haplotypes and diplotypes were inferred using the Expectation-Maximization algorithm. Haplotype H5 was significantly associated with reduced NSCLC risk in former smokers with an OR of 0.47 (0.25–0.96) compared with the common H1 haplotype. Compared with the H1–H2 diplotype, H2–H2 and H3–H4 diplotypes were associated with increased NSCLC risk with ORs of 1.58 (0.99–2.54) and 2.29 (1.05–5.00), respectively. We then evaluated genotype–phenotype correlation in the control group using the comet assay to determine DNA damage and DNA repair capacity. Compared with individuals with at least 1 wild-type allele, the homozygous variant carriers of either *ATM*08 or *ATM*10 exhibited significantly increased DNA damage as evidenced by a higher mean value of the radiation-induced olive tail moment (*ATM*08: 4.86 ± 2.43 *vs.* 3.79 ± 1.51, $p = 0.04$; *ATM*10: 5.14 ± 2.37 *vs.* 3.79 ± 1.54, $p = 0.01$). Our study presents the first epidemiologic evidence that *ATM* genetic variants may affect NSCLC predisposition, and that the risk-conferring variants might act through down-regulating the functions of ATM in DNA repair activity upon genetic insults such as ionizing radiation.

Keywords

ATM; polymorphism; haplotype; diplotype; NSCLC

Lung cancer accounts for 20% of cancer incidence in the United States and 25% of cancer-related deaths. Approximately 80% of lung cancer cases are non-small cell lung cancer (NSCLC). Deficiencies in DNA repair capacity (DRC), apoptosis control and cell cycle checkpoints have been implicated in the pathogenesis of lung cancer including NSCLC.[1–3] Therefore, the essential role of the ATM protein in double strand break (DSB) DNA damage

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response and the importance of the DNA repair system in tobacco-related carcinogenesis highlight the potential significance of *ATM* sequence variations on NSCLC risk.

ATM is a tumor suppressor gene frequently mutated in patients with Ataxia Telangiectasia (AT), a rare form of an autosomal recessive malignancy-prone disorder prominently characterized by extremely high sensitivity to ionizing radiation or other DSB-inducing agents.[4] *ATM* encodes a 370-kDa phosphoinositide 3-kinase (PI3K) protein that belongs to the PI3K-like Serine/Threonine protein kinase (PIKK) family. This family functions in DNA damage responses by phosphorylating proteins in various damage-related pathways.[5] ATM exists in an inactive multimer form in the cell nucleus, which dissociates into monomers upon exposure to DSB-inducing genetic insults.[6] The interaction between the MRN (MRE11, RAD50, NBS1) complex and ATM in the presence of damaged DNA yields a more than 80-fold increase in ATM kinase activity, which is capable of relating the signals to a plethora of downstream effectors through phosphorylation of specific serine or threonine amino acid residues.[7] The converging effects of these protein effectors control the outcome of the damaged cells through the regulation of cell cycle arrest, DNA repair and apoptosis.

Aberrations of ATM protein have been implicated in the etiology of many cancers,[8–13] including lung cancer. Through phosphorylating p53 and MDM2 proteins, ATM disrupts the p53-MDM2 interaction and thus, increases the nuclear accumulation of p53.[5] Consistent with this notion, Bartkova *et al.* found that ATM, CHK2, p53 and H2AX were highly expressed and phosphorylated in early precursor lesions of various cancers, suggesting that the ATM-CHK2-p53 axis plays an essential role in the DNA damage-response in the early development stage of these malignancies, including lung cancer.[14] Moreover, Eymin *et al.* reported that the ATM/CHK2 pathway also mediates the $p14^{ARF}$-induced G2 cell cycle checkpoint arrest in response to DNA damaging agents. This pathway is independent of p53 activation and its defects contribute to lung carcinogenesis.[14] Taken together, these observations highlight the pivotal role of ATM in the prevention of lung cancer development through the modulation of multiple pathways. However, most previous studies focused on the carcinogenic effect of *ATM* rare mutations rather than common variants. In a few studies in which *ATM* polymorphisms were investigated, controversial results have been reported in terms of the involvement of *ATM* polymorphisms in the etiology of malignancies such as breast and colorectal cancers.[15–20] There have not been any studies of *ATM* polymorphisms and lung cancer risk in Caucasians. In addition, although *ATM* haplotypes have also been associated with altered cancer risk, most published studies focused on only breast cancer and the haplotypes in these studies were composed of only potential functional SNPs but were not based on haplotype tagging SNPs (htSNP).[15,21–23] Since Bonnen *et al.* reported extensive linkage disequilibrium (LD) across the complete *ATM* locus which suggested that few htSNPs were required to construct complete high-power haplotypes to capture common *ATM* polymorphisms,[24] a comprehensive approach combining the power of htSNPs and functional SNPs may provide more clues to the assessment of *ATM* sequence variants on cancer risk.

To test the hypothesis that common *ATM* sequence variants may modulate NSCLC risk, we assessed the associations of 11 potential *ATM* htSNPs and functional SNPs with NSCLC risk in Caucasians. In addition, we performed a functional assay to evaluate the physiological significance of the observed associations through genotype–phenotype correlation analyses. To the best of our knowledge, this is the first epidemiological study examining the role of *ATM* polymorphisms in NSCLC risk in Caucasians.

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Material and methods

Study population

Lung cancer cases were recruited from The University of Texas MD. Anderson Cancer Center. Cases were newly diagnosed and histologically confirmed lung cancer patients who had received no previous chemotherapy or radiotherapy. There were no recruitment restrictions on age, gender, ethnicity or cancer-stage. The controls were recruited from the Kelsey-Seybold Clinic, Houston's largest private multispecialty physician group which includes a network of 23 clinics and more than 300 physicians. Potential controls were identified from healthy individuals without a previous diagnosis of cancer except for nonmelanoma skin cancer. We excluded subjects who had recent blood transfusions to control for confounding effects for several functional assays. Potential controls were first surveyed by a short questionnaire for willingness to take part in case-control studies and to provide demographic and smoking status data for matching. Controls were frequency-matched to cases in terms of age (±5 years), gender, ethnicity and smoking status. Definition criteria of smoking status were as previously described.[2] For both cases and controls, after obtaining written informed consent, trained M.D. Anderson staff interviewers administered risk factor questionnaires to study participants. The interview took ~45 min to complete. Data were collected on demographic characteristics (age, gender, ethnicity, *etc.*), work history, tobacco use history and family history of cancer. This case-control study started in 1995 and is currently ongoing. The response rate of participation is ~77.4% for cases and 73.3% for controls. Participants who had blood transfusions within recent 6 months were excluded. At the completion of the interview, 40 ml of blood were drawn from each person and sent to the laboratory for DNA isolation and molecular analysis. Laboratory personnel were blinded to case and control status. Human subject approval was obtained from the institutional review boards of both M.D. Anderson and Kelsey–Seybold. In the current analysis, a total of 556 Caucasian NSCSL patients and 556 cancer-free controls (frequency-matched by age, gender and smoking status) were included.

Selection of ATM sequence variants

Eleven potential haplotype-tagging and functional SNPs of the *ATM* gene ranging from 10-kb upstream of the translation initiation site to 5-kb downstream of the translation stop site were chosen based on the data currently available from public SNP databases, including NCBI dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP), International HapMap Project (http://www.hapmap.org) and Cancer Genome Anatomy Project SNP500Cancer (http://snp500cancer.nci.nih. gov). To choose potential htSNP sets, we used the SNPbrowser 3.5 software (Applied Biosystems, Foster, CA) implementing the pairwise r^2 algorithms. The remaining SNPs with higher than 5% minor allele frequency (MAF) were selected empirically after exhaustively searching the relevant literature to locate potential functional *ATM* SNPs that had been implicated in other cancer association studies.

Probe and primer designs and genotyping

Genotyping was performed using a 5′ nuclease assay-based *TaqMan* assay. Probes and primers for the genotyping were either acquired from the SNP500Cancer database or designed using the PrimerExpress 2.0 software (Applied Biosystems, Foster, CA). The probes were labeled fluorescently with either 6-FAM or VIC on the 5′ end and a nonfluorescent minor groove binder (MGB) quencher on the 3′ end. The genotyping procedure was exactly as described in a previous study.[25] Genomic DNA was extracted from peripheral blood lymphocytes using the Human Whole Blood Genomic DNA Extraction Kit (Qiagen, Valencia, CA). The PCR amplification mix (5 μl) included sample DNA (5 ng), 1 X *TaqMan* buffer A, 200 μM deoxynucleotide triphosphates, 5 mM $MgCl_2$, 0.65 U of AmpliTaq Gold, 900 nM each primer and 200 nM each probe. The PCR condition

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includes 1 cycle for 10 min at 95°C, 40 cycles for 15 sec at 95°C and 1 min at 60°C. PCR was performed using ABI PRISM® 7900HT sequence detection system (Applied Biosystems) and SDS 2.1 software (Applied Biosystems) was used to analyze the end-point genotyping data. Internal quality controls and negative controls were used to ensure genotyping accuracy and 5% of all samples were randomly selected and genotyped in duplicates with 100% concordance.

Comet assay

Baseline and γ-radiation-induced comet assays were performed in the control group as described previously.[26] The comet assays in this study were performed following exactly the same experimental procedures and using the same software. All reagents were purchased from the same vendor and freshly made. Briefly, fully frosted, agarose-coated slides were covered with a glass coverslip and left at room temperature for 30 min. Blood cultures were either untreated or irradiated with 1.5 Gy using a ^{137}Cs source at room temperature. This dose was reported in our previous studies to be the optimal dose, which is sufficient to induce nuclear DNA damage but is not cytotoxic.[26] The untreated or γ-irradiated blood culture was mixed with low melting point (LMP) agarose (Invitrogen, Carlsbad, CA) in PBS. About 50-μl mixture was immediately spread onto each end of the slide, covered with a fresh coverslip, and left at 4°C for 10 min. The slides were submersed in freshly prepared lysis buffer for 1 hr at 4°C and placed in a horizontal electrophoresis box without power and filled with freshly prepared alkali for 30 min at 4°C. After an electrophoresis at 295–300 mA for 23 min at 4°C, the slides were neutralized in Tris buffer, fixed in 100% methanol for 10 min and stored in the dark at room temperature. Immediately before analysis, slides were hydrated in fresh Tris–HCl and then stained with ethidium bromide. Fifty consecutive cells (25 cells from each end of the slide) were manually selected and quantified with Komet version 4.0.2 (Kinetic Imaging, Bromborough, Wirral, UK) software attached to a fluorescent microscope (Nikon, Melville, NY), which also determined the Olive tail moment parameter [(tail mean − head mean) × (% tail DNA/100)]. The head of the comet represents the cell nucleus and the tail of the comet represents the damaged DNA liberated from the nucleus by electrophoresis. The tail mean is the tail DNA intensity subtracted by background intensity, while the head mean is the head DNA intensity subtracted by background intensity. The percentage of tail DNA is the fraction of DNA that has migrated from the head. The difference between the tail mean and the head mean represents the difference in the distance between the center of gravity of the DNA distribution in the comet head and the center of gravity of the DNA distribution in the comet tail. Since the comet assay was not started from the beginning of the subject recruitment, data was not available for those study subjects who were enrolled before the commencement of the comet assay. In the current analysis, there were 116 controls subjects with baseline comet data. Among them, 112 had γ-radiation-induced comet assay data. There are no significant differences in major host characteristics between the control subjects with comet data and those without comet data (data not shown).

Statistical analysis

Statistical analyses were done using either SAS software (SAS Institute Cary, CA) or Intercooled Stata 8.0 statistical software package (Stata, College Station, TX). χ^2 and Fisher's exact tests were used to assess patient characteristics. The risks were calculated as odds ratios (OR) and 95 percent confidence intervals (95% CI) using unconditional multivariate logistic regression adjusted by age, gender and smoking status (never, former and current smokers). We analyzed the associations between individual SNPs and NSCLC risk by combining the genotypes with at least 1 wild-type allele as the reference group as *ATM* is a tumor suppressor gene that might function in a recessive pattern. Haplotypes and diplotypes were estimated using the Expectation-Maximization (EM) algorithm

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implemented by the HelixTree program (Golden Helix, Bozeman, MT). Haplotypes with a probability less than 95% were excluded from the final data to ensure analytical reliability.[15] A two sample *t* test with equal variance was used to determine the genotype–phenotype correlation. $p \leq 0.05$ was considered as the threshold of significance. All statistical analyses were 2-sided.

Results

Individual ATM polymorphisms and NSCLC risk

Table I lists the selected characteristics of the study population and illustrates that the case patients and control subjects were well matched on age, gender and smoking status. The average genotyping call rate for all SNPs is 97.4 % (95.8–98.9%) and there are no significant call rate differences between cases and controls (the average call rate is 97.6 and 97.3% for cases and controls, respectively).

Table II summarizes the name, reference number, gene position and genotypic distributions of each SNP and their associations with NSCLC risk. Two highly linked intronic SNPs, *ATM*08 and *ATM*10, exhibited significant and borderline significant associations with NSCLC risk, respectively, when their homozygous wild-type plus heterozygous genotypes were used as the reference group. For the *ATM*08 polymorphism, when compared to the wild-type CC genotype, carriers of 1 variant allele (CT) showed a nonsignificant protective effect (OR = 0.79 (0.61–1.02)) whereas the homozygous variant genotype (TT) exhibited a nonsignificant association with an increased NSCLC risk (OR = 1.38 (0.89–2.13)) (data not shown). However, when the genotypes of at least 1 wild-type allele were combined together as the reference group (CC + CT), the TT genotype demonstrated a significant association with an increased NSCLC risk (OR = 1.55 (1.02–2.35)). Similarly, when the group with at least 1 wild-type allele of *ATM*10 was used as a reference (AA + AC), the CC genotype showed a borderline statistically significant association with an elevated risk (OR = 1.51 (0.99–2.31)).

The manifested risk of both SNPs were more evident in males, in young people (≤61 years old), and in former smokers with ORs of 1.87 (1.08–3.26) 2.32 (1.26–4.27) and 2.01 (1.05–3.84) for *ATM*08 respectively, and ORs of 1.84 (1.04–3.24), 2.09 (1.13–3.88) and 2.07 (1.08–3.94) for *ATM*10 respectively (Table III). The double-variant genotypes of other SNPs including *ATM*01, *ATM*02, *ATM*05, *ATM*06 *ATM*07, *ATM*09 and *ATM*11 did not exhibit apparent alterations in ORs. The small sample size of the homozygous-variant genotypes of *ATM*03 and *ATM*04 limited their further analysis (Table II).

Associations of ATM haplotypes with NSCLC risk

Eight haplotypes with frequency greater than 1% in both cases and controls were identified. Six common haplotypes accounted for 98% of the total haplotypes. Other software implemented with Bayesian algorithm[27] produced very similar results (data now shown). The most common haplotype H1 (TTCCGTCCGAT) (in the order from *ATM*01 to *ATM*11) showed a frequency of 36% in both cases and controls. There was no overall association between haplotypes and NSCLC risk using the most common H1 haplotype as the reference group (Table IV). However, on stratified analysis, the H5 haplotype (TTCTGTCCGAT) was significantly associated with a decreased risk in former smokers (OR = 0.47 (0.25–0.96)) (Table IV).

Associations of ATM diplotypes with NSCLC risk

Diplotypes were reconstructed using the estimated haplotypes with probability greater than 95% (Table V). The 10 highest diplotypes constituted more than 86% of the cases and 86%

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of the controls. Compared to the most common diplotype (H1–H2), H2–H2 manifested a borderline significant association with increased NSCLC risk (OR = 1.58 (0.99–2.54)). The effect was more prominent in young people with an OR of 2.26 (1.13–4.52) and in former smokers with an OR of 2.22 (1.06–4.65) (data not shown). The increased risk associated with H2–H2 was in line with the result of the individual significant SNPs since H2–H2 was the only diplotype containing homozygous variant alleles of both *ATM*08 and *ATM*10. The H3–H4 diplotype also exhibited a significant risk effect (OR = 2.29 (1.05–5.00)), which was higher in females (OR = 8.17 (1.69–39.33)) (data not shown). The H2–H4 diplotype did not demonstrate overall significance; however, it showed a significant effect within the strata of females (OR = 2.85 (1.03–7.79)) and never smokers (OR = 3.94 (1.04–14.93)) (data not shown).

Genotype–phenotype correlations in controls

To validate the biological implication of the demonstrated associations between increased NSCLC risk and *ATM*08/*ATM*10, we performed genotype–phenotype correlation analyses in 116 control subjects with available comet data. The baseline comet assay showed no noteworthy distinctions between the reference group and the homozygous variants (Table VI). However, individuals with homozygous variant genotypes demonstrated significantly elevated γ-radiation-induced DNA damage, as represented by higher levels of olive tail moment, compared to carriers of at least 1 wild-type allele in either *ATM*08 or *ATM*10 (*ATM*08, TT *vs.* CC+CT: 4.86 ± 2.43 *vs.* 3.79 ± 1.51, $p = 0.04$; *ATM*10, CC *vs.* AA + AC: 5.14 ± 2.37 *vs.* 3.79 ± 1.54, $p = 0.01$) (Table VI). The average interassay coefficient variation for the γ-radiation comet assay was 9.8% (data not shown), which is modest compared with the variation resulting from genotype differences of *ATM*08 and *ATM*10.

Discussion

The main finding of this report is that two-linked intronic SNPs of *ATM* exhibited a similar recessive pattern of association with increased NSCLC risk, which is strongly supported by the comet assay indicating that the variants might function through influencing the DNA damage/repair pathway of the host cell. No literature has investigated the associations of these 2 SNPs with any form of cancer or other diseases. The close proximity of the 2 SNPs (in introns 61 and 62) to the *ATM* PI3 kinase domain (exons 60 and 61) lends support to the hypothesis that these 2 SNPs might regulate ATM PI3 kinase activity by affecting either the kinase domain or FAT domain immediately before the kinase motif.

All SNPs conformed to Hardy–Weinberg Equilibrium (HWE) in both case patients and control subjects, except for *ATM*08 and *ATM*10, which showed significant deviation in controls (Table II, 0.02 and 0.04, respectively). However, the divergence from HWE is not likely the result of genotyping errors since we observed complete concordance from genotyping quality controls. Moreover, neither of the 2 SNPs were still out of HWE when the Bonferroni-corrected significance threshold was applied to test the null hypothesis of HWE to detect genotyping errors.[28] Similar results were obtained when we used the less conservative Benjamini–Hochberg step-up procedure to control for false discovery rate (FDR) of HWE at the 5% level (data not shown). More importantly, the results of our comet functional assays measuring the DNA damage/DRC indicated that the homozygous variant genotype of these 2 SNPs were associated with significantly higher levels of gamma-radiation-induced DNA damage, strongly suggesting that the elevated risk associated with them are biologically plausible rather than being attained by population selection bias.

Validation through functional assays lends supports to the conclusions drawn from genotyping data. However, such assays are scarce in most published cancer association studies. In the present report, we performed the comet assay, a single cell gel

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electrophoresis-based laboratory test frequently used to evaluate DNA damage/repair and genotoxicity,[29] to assess the correlation of *ATM* genotypes with the host DNA damage/repair capacity in the control group. We discovered that radiation-induced-, but not baseline-, DNA damage was significantly higher in the homozygous variant genotypes of both *ATM*08 and *ATM*10, when compared to genotypes with at least 1 wild-type allele. This was strongly consistent with the role played by ATM in monitoring radiation-induced DSB DNA damage and initiating the corresponding repair process.[30] The delicate structure of the ATM protein has remained elusive, which has limited in-depth investigation of the interactions between ATM and relevant signal effectors implicated in DSB repair. It is clear that one of the converging effects of the ATM-mediated signal pathways is to induce cell cycle arrest and allow the cell to repair the damage. Therefore, the hypothesis that *ATM* functional variants influence the function of the ATM protein on DNA repair coincide with the phenotypic assay results that the samples with homozygous variants of either *ATM*08 or *ATM*10 showed a significant increase in DNA damage as well as increased NSCLC risk. This finding was further supported by the data showing the associations of diplotype H2–H2, the only analyzed diplotype containing the homozygous variants of both *ATM*08 and *ATM*10, with elevated NSCLC risk. Previous reports have suggested that most mutations/variations in *ATM* region exert aberrant functions by influencing ATM activity instead of its protein expression.[31,32] Analysis of kinase activity of Ser^{1981}-phosphorylated ATM,[6] as well as the expression profile of the total ATM level using peripheral blood cells derived from subjects with different *ATM*08/*ATM*10 genotypes will cast more light on this hypothesis.

No significant gene-dose effect was identified for either *ATM*08 or *ATM*10. This may be because *ATM* is a tumor suppressor gene and, thereby, 2 copies of the gene need to be inactivated before carcinogenesis ensues. This recessive functional fashion is a common theme of many tumor suppressor genes and the “two-hit” hypothesis of carcinogenesis is the most convincing explanation for its working mechanism.[33] We also noticed that the elevated risks for *ATM*08 and *ATM*10 were higher in former smokers than in current smokers. An unambiguous hypothesis regarding interaction between smoking and *ATM* genetic variants has yet to be developed. The genetic effects of *ATM* variants observed in this study may have been overwhelmed by the strong smoking exposure in current smokers. Moreover, as the results of the stratified analyses were based on relatively small sample size, this result needs to be interpreted with caution and requires validation in larger studies. Among the 6 intronic SNPs analyzed, *ATM*02, *ATM*07 and *ATM*09 have been associated with risk of breast cancer[15,16]; however, our data did not suggest that any of these was related to NSCLC risk. *ATM*05, the only nsSNP currently identified as having an MAF ≥10%, has been implicated in various types of cancer[18–20] but showed no effect in this study. Furthermore, haplotypes constructed using only potential functional SNPs (nsSNPs and regulatory SNPs, including *ATM*01, *ATM*03, *ATM*05 and *ATM*11) did not reveal any association with NSCLC risk (data not shown).

In a recent case-control study, Kim *et al.* described the association of rs664143 (*ATM*09) with increased lung cancer risk in a Korean population.[22] This SNP did not show an evident link with NSCLC risk in our study, possibly due to the remarkable difference in the allelic frequency between these 2 populations.[22] In addition, we restricted our case patients to NSCLC, whereas Kim *et al.* investigated all lung cancer subtypes.

In summary, our study presents the first epidemiological data describing the associations of 2 common *ATM* polymorphisms (*ATM*08 and *ATM*10) and elevated NSCLC risk. The hypothesis that these 2 SNPs function through influencing ATM kinase activity was underscored by the independent phenotypic assay analyzing DNA repair profiles. Nevertheless, we cannot rule out the possibility that other rare *ATM* polymorphisms in LD

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with these 2 SNPs are the real causative agents. Further investigations exploring the molecular mechanism of the proposed *ATM* functional variations as well as the downstream signal transducers mediating the observed effects are warranted to provide a new dimension to our current understanding of the significance of *ATM* sequence variants in a more inclusive picture of lung carcinogenesis.

Acknowledgments

Grant sponsor: NCI; Grant numbers: CA 111646, CA 55769, CA 70907, DAMD17-02-1-0706; Grant sponsor: Flight Attendant Medical Research Institute.

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TABLE I

DISTRIBUTION OF SELECTED HOST CHARACTERISTICS BY CASE-CONTROL STATUS

Variable	Case, *N* (%)	Control, *N* (%)	*p* value[1]
Age (Mean ± SD[2])	60.5 ± 10.6	60.1 ± (10.3)	0.48
Gender			
Male	309 (55.6)	309 (55.6)	
Female	247 (44.4)	247 (44.4)	1.00
Smoking status			
Never	94 (16.9)	106 (19.1)	
Former	219 (39.4)	236 (42.4)	
Current	243 (43.7)	214 (38.5)	0.20[3]
Ever	462 (83.1)	450 (80.9)	0.35[4]
Pack-years (Mean ± SD)	50.6 ± 29.1	47.3 ± 32.1	0.11

[1] *p* values were derived from the χ^2 test for categorical variables (gender and smoking status) and *t* test for continuous variables (age and pack-years). –

[2] SD, standard deviation.

[3] Among never, former and current smokers.–

[4] Ever smokers compared with never smokers.

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TABLE II

CASE-CONTROL DISTRIBUTION OF GENOTYPES OF 11 POTENTIAL *ATM* SEQUENCE VARIANTS

	Reference	Position	Genotypes	Cases, *N* (%)	Controls, *N* (%)	OR[1] (95% CI[1])[2]	HWE[3] *p* (controls)
*ATM*01	rs228589	5′ region	AA + AT	434 (80.7)	433 (80.8)	1.00 (reference)	0.31
			TT	104 (19.3)	103 (19.2)	1.01 (0.74–1.37)	
*ATM*02	rs664677	Intron	TT + TC	445 (81.4)	441 (81.7)	1.00 (reference)	0.34
			CC	102 (18.6)	99 (18.3)	1.03 (0.81–1.14)	
*ATM*03	rs1800058	Exon, nsSNP[4]	CC + CT	549 (99.8)	550 (100)	1.00 (reference)	0.60
			TT	1 (0.2)	0 (0)	N/A[5]	
*ATM*04	rs1800889	Exon, sSNP[4]	CC + CT	548 (100)	550 (99.8)	1.00 (reference)	0.80
			TT	0 (0)	1 (0.2)	N/A	
*ATM*05	rs1801516	Exon, nsSNP	GG + GA	537 (98.7)	536 (98.2)	1.00 (reference)	0.59
			AA	7 (1.3)	10 (1.8)	0.70 (0.26–1.85)	
*ATM*06	rs611646	Intron	TT + TA	445 (81.4)	458 (85.0)	1.00 (reference)	0.18
			AA	102 (18.6)	81 (15.0)	1.30 (0.95–1.80)	
*ATM*07	rs609429	Intron	GG + GC	436 (80.9)	438 (81.4)	1.00 (reference)	0.25
			CC	103 (19.1)	100 (18.6)	1.04 (0.76–1.41)	
*ATM*08	rs227060	Intron	CC + CT	469 (88.7)	495 (92.4)	1.00 (reference)	0.02
			TT	60 (11.3)	41 (7.6)	**1.55 (1.02–2.35)**	
*ATM*09	rs664143	Intron	GG + GA	433 (80.6)	441 (82.3)	1.00 (reference)	0.16
			AA	104 (19.4)	95 (17.7)	1.12 (0.82–1.52)	
*ATM*10	rs170548	Intron	AA + AC	483 (89.3)	500 (92.6)	1.00 (reference)	0.04
			CC	58 (10.7)	40 (7.4)	**1.51 (0.99–2.31)**	
*ATM*11	rs4585	3′ region	TT + TG	442 (80.8)	440 (81.5)	1.00 (reference)	0.14
			GG	105 (19.2)	100 (18.5)	1.05 (0.77–1.42)	

The bold type face indicates a significant results.

[1] OR, odds ratio; CI, confidence interval.

[2] Adjusted for age, gender and smoking status.

[3] HWE, Hardy–Weinberg equilibrium.

[4] nsSNP, non-synonymous SNP; sSNP, synonymous SNP.

[5] N/A, not available.

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TABLE III

CASE-CONTROL DISTRIBUTIONS OF *ATM*08 AND *ATM*10 STRATIFIED BY SELECT VARIABLES

	*ATM*08				*ATM*10		
	Cases, *N* (%)	**Controls, *N* (%)**	**Adjusted, OR (95% CI)[1]**		**Cases, *N* (%)**	**Controls, *N* (%)**	**Adjusted, OR (95% CI)**
Age							
<61[2]							
CC + CT	233 (83.8)	231 (81.1)	1.00 (reference)	AA + AC	242 (88.3)	265 (94.0)	1.00 (reference)
TT	45 (16.2)	54 (18.9)	**2.32 (1.26–4.27)**	CC	32 (11.7)	17 (6.0)	**2.09 (1.13–3.88)**
≥61							
CC + CT	236 (90.1)	228 (90.5)	1.00 (reference)	AA + AC	241 (90.3)	235 (91.1)	1.00 (reference)
TT	26 (9.9)	24 (9.5)	1.05 (0.59–1.89)	CC	26 (9.7)	23 (8.9)	1.12 (0.62–2.02)
Sex							
Male							
CC + CT	256 (87.1)	275 (92.6)	1.00 (reference)	AA + AC	265 (88.0)	246 (92.1)	1.00 (reference)
TT	38 (12.9)	22 (7.4)	**1.87 (1.08–3.26)**	CC	36 (12.0)	21 (7.9)	**1.84 (1.04–3.24)**
Female							
CC + CT	213 (90.6)	220 (92.1)	1.00 (reference)	AA + AC	218 (90.8)	221 (92.1)	1.00 (reference)
TT	22 (9.4)	19 (7.9)	1.17 (0.61–2.23)	CC	22 (9.2)	19 (7.9)	1.16 (0.61–2.21)
Smoking status							
Never smoker							
CC + CT	84 (92.3)	97 (91.5)	1.00 (reference)	AA + AC	96 (93.2)	94 (91.3)	1.00 (reference)
TT	7 (7.7)	9 (8.5)	0.89 (0.32–2.49)	CC	7 (6.8)	9 (8.7)	0.85 (0.30–2.38)
Former smoker							
CC + CT	182 (86.7)	207 (92.8)	1.00 (reference)	AA + AC	184 (86.8)	215 (93.1)	1.00 (reference)
TT	28 (13.3)	16 (7.2)	**2.01 (1.05–3.84)**	CC	28 (13.2)	16 (6.9)	**2.07 (1.08–3.94)**
Current smoker							
CC + CT	203 (89.0)	191 (92.3)	1.00 (reference)	AA + AC	213 (90.3)	191 (92.7)	1.00 (reference)
TT	25 (11.0)	16 (7.7)	1.46 (0.75–2.82)	CC	23 (9.7)	15 (7.3)	1.36 (0.69–2.69)

[1] Adjusted for age, gender and smoking status.

[2] 61 is the mean age of control subjects.

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TABLE IV

DISTRIBUTION OF *ATM* HAPLOTYPES AND NSCLC RISK IN CASE PATIENTS AND CONTROL SUBJECTS BY SMOKING STATUS

Haplotypes sequence	Overall			Never smoker			Former smoker			Current smoker		
	Cases, *N* (%)	Controls, *N* (%)	OR (95% CI)[1]	Cases, *N* (%)	Controls, *N* (%)	OR (95% CI)	Cases, *N* (%)	Controls, *N* (%)	OR (95% CI)	Cases, *N* (%)	Controls, *N* (%)	OR (95% CI)
H1 TTCCGTCCGAT	388 (36.3)	397 (36.6)	1.00 (reference)	63 (34.6)	92 (43.8)	1.00 (reference)	157 (36.9)	154 (33.3)	1.00 (reference)	168 (36.4)	151 (36.7)	1.00 (reference)
H2 ACCCGAGTACG	334 (31.2)	338 (31.2)	1.01 (0.82–1.24)	55 (30.2)	67 (31.9)	1.17 (0.72–1.90)	133 (31.2)	144 (31.2)	0.91 (0.66–1.27)	146 (31.6)	127 (30.8)	1.03 (0.74–1.42)
H3 ACCCATGCAAG	160 (15.0)	156 (14.4)	1.04 (0.80–1.35)	27 (14.8)	22 (10.5)	1.81 (0.95–3.48)	66 (15.5)	69 (14.9)	0.96 (0.64–1.44)	67 (14.5)	65 (15.8)	0.93 (0.62–1.40)
H4 ACCCGAGCAAG	102 (9.5)	99 (9.1)	1.06 (0.78–1.45)	20 (11.0)	16 (7.6)	1.93 (0.91–4.05)	38 (8.9)	49 (10.6)	0.77 (0.48–1.25)	44 (9.5)	34 (8.3)	1.15 (0.70–1.90)
H5 TTCTGTCCGAT	40 (3.7)	53 (4.9)	0.76 (0.49–1.18)	10 (5.5)	6 (2.9)	2.24 (0.75–6.60)	13 (3.1)	27 (5.8)	**0.47 (0.25–0.96)**	17 (3.7)	20 (4.9)	0.79 (0.40–1.57)
H6 TTTCGTCCGAT	33 (3.1)	24 (2.2)	1.47 (0.85–2.54)	5 (2.7)	6 (2.9)	1.38 (0.39–4.84)	13 (3.1)	11 (2.4)	1.23 (0.53–2.85)	15 (3.2)	7 (1.7)	2.00 (0.79–5.06)
Others[2]	13 (1.2)	17 (1.6)	0.76 (0.36–1.59)	2 (1.1)	1 (0.5)	2.18 (0.19–25.5)	6 (1.4)	8 (1.7)	0.72 (0.24–2.13)	5 (1.1)	8 (1.9)	0.59 (0.19–1.85)

[1] Adjusted for age, gender and smoking status.

[2] This category combined all other haplotypes.

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TABLE V

ATM DIPLOTYPES AND NSCLC RISK

Diplotype	Cases *N* (%)	Controls *N* (%)	OR (95% CI)[1]
H1–H2	120 (22.4)	137 (25.3)	1.00 (reference)
H1–H1	72 (13.5)	68 (12.5)	1.21 (0.80–1.83)
H1–H3	61 (11.4)	56 (10.3)	1.23 (0.79–1.91)
H2–H2	58 (10.8)	41 (7.6)	**1.58 (0.99–2.54)**
H2–H3	46 (8.6)	55 (10.1)	0.92 (0.58–1.47)
H1–H4	32 (6.0)	34 (6.3)	1.05 (0.61–1.81)
H2–H4	30 (5.6)	34 (6.3)	1.02 (0.58–1.77)
H3–H4	21 (3.9)	11 (2.0)	**2.29 (1.05–5.00)**
H2–H5	14 (2.6)	17 (3.1)	0.90 (0.42–1.93)
H1–H5	13 (2.4)	16 (3.0)	0.94 (0.43–2.05)
Others[2]	68 (12.7)	73 (13.5)	1.08 (0.71–1.63)

[1] Adjusted for age, gender and smoking status.

[2] This category combines all other diplotypes.

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Int J Cancer. Author manuscript; available in PMC 2012 October 22.

TABLE VI

ASSOCIATIONS OF GENOTYPES OF *ATM*08 AND *ATM*10 WITH DNA DAMAGE/REPAIR ASSESSED BY COMET ASSAY IN CONTROL SUBJECTS

	Baseline			γ-radiation-induced		
	N	Mean ± SD[1]	*p* value[2]	*N*	Mean ± SD	*p* value
ATM08						
CC + CT	103	1.38 ± 0.70		99	3.79 ± 1.51	
TT	11	1.39 ± 0.79	0.96	11	4.86 ± 2.43	**0.04**
ATM10						
AA + AC	101	1.39 ± 0.72		97	3.79 ± 1.54	
CC	10	1.43 ± 0.80	0.85	10	5.14 ± 2.37	**0.01**

[1] SD, standard deviation.

[2] *p* value for *t* test.