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Original Article

APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population

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Contributions: (I) Conception and design: CXJ, GBQ; (II) Administrative support: XSB, DT, JYL, MW; (III) Provision of study materials or patients: XSB, DT; (IV) Collection and assembly of data: JYL, MW, FX, JLZ, JMC, QYF; (V) Data analysis and interpretation: JYL, MW, XQW, SL, XX, YTY; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract

Background: Approximately 80–85% of lung cancer is the non-small cell lung cancer (NSCLC) subtype, which ranks as the leading cause of cancer deaths worldwide. *APOBEC3B* (A3B) was reported to be a key source of mutations in NSCLC. However, the role of the A3B deletion polymorphism in the etiology of NSCLC has not been well-documented.

Methods: A case-control study with 317 NSCLC patients and 334 healthy controls was conducted to explore the association between the A3B deletion polymorphism and the risk of NSCLC. The unconditional logistic regression model was performed to calculate the odds ratio (OR) and the 95% confidence interval (CI), and the confounding factors were adjusted, including age, gender, and smoking status, to estimate the risk. An analysis of gene-environment interactions was performed using multifactor dimensionality reduction (MDR) software.

Results: We found that the del/del genotype of A3B deletion significantly increased NSCLC risk. Compared with individuals carrying the ins/ins genotype of A3B deletion, individuals with the del/del genotype had a 2.36 times increased risk of developing NSCLC after adjusting for confounding factors (OR =2.71, 95% CI: 1.67–4.42, P<0.001). A 3-factor gene-environment (A3B deletion, gender, and smoking) interaction model was found for NSCLC (OR =4.407, 95% CI: 1.174–16.549, P=0.028).

Conclusions: We propose that the A3B deletion polymorphism can increase the risk of developing NSCLC, and their interactions with gender and smoking may contribute to the risk of NSCLC in the southern Chinese population.

Keywords: Non-small cell lung cancer (NSCLC); APOBEC3B deletion (A3B deletion); polymorphism; interaction; association

#Introduction

Lung cancer ranks as the most commonly diagnosed cancer. and the leading cause of cancer death worldwide, with an estimated 2,093,876 new cases and 1,761,007 deaths

in 2018 (1). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the 2 main histological subtypes of lung cancer, and approximately 80–85% of lung cancers are NSCLC, including adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma (2). Although medical technology has developed rapidly and has significantly improved the survival of lung cancer patients, the etiology of lung cancer remains unclear and prognosis is still poor for some patients, necessitating further investigations. Smoking, as one of the most well-known environmental risk factors, is a major contributor to lung cancer development. According to the Global Burden of Disease, in 2017, approximately 63% of lung cancer deaths in China were attributable to smoking (3). Furthermore, there is also a genetic component to lung cancer (4), and heritability estimates of the genetic risk for lung cancer are approximately 8% (5). Smoking can also cause gene mutations which may lead to a heavy mutation load of lung cancer (6, 7). Therefore, while smoking is a clear risk factor, the genetic factors also cannot be ignored.

The apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (A3) family (8), which plays a crucial role in antiviral innate cellular immunity, consists of 7 members [A3A, *APOBEC3B* (A3B), A3C, A3DE, A3F, A3G, and A3H] on chromosome 22 (9). They can cause deaminations of cytosine to uracil in DNA via an enzyme called activation-induced cytidine deaminase (10), leading to DNA damage. Studies have shown that the activity of A3A and A3B are linked to cancer. Recent research has also shown that the expression levels of A3B have a strong positive correlation with tumor mutation loads (11), along with a strong A3B upregulation in the majority of tumor types, including breast, uterus, bladder, head and neck, and lung (adeno- and squamous cell carcinomas) (12), suggesting that A3B may be a general endogenous mutagen that contributes to human cancers. Patients with a higher level of A3B gene expression in their tumors had a lower survival rate as compared to patients with lower expression A3B gene expression in their tumors(13-15).

Recently, a 29.5 kb germline deletion in the A3 gene region has been found to be associated with the risk of breast cancer (11), colon cancer (16), cervical cancer (17), lung cancer (16), prostate cancer (16), ovarian cancer (18), bladder cancer (19), oral cancer (17), and hepatocellular carcinoma (20). This 29.5 kb deletion removes the 3' end of the A3A gene and a large part of the A3B gene, creating a hybrid gene that

transcribes mRNA with the A3A coding region and the A3B 3′-untranslated regions (UTR) (19), and the amino acid sequence of this fusion protein is identical to APOBEC3A (21). Interestingly, carriers of the A3B deletion transcript were shown to have higher A3A mRNA stability, resulting in higher intracellular levels and more severe DNA damage (22). Among all the members of the A3 family, A3A can hypermutate DNA by inducing DNA double-strand breaks (23, 24), suggesting A3A has a role in pro-inflammatory conditions that might cause cancer (25). The *A3B* germline deletion allele also causes deletion of the A3B coding sequence, and thus, the absence of A3B in homozygous patients (21). Evidence also has supported the association between A3B upregulation and NSCLC-related processes (26). However, the mechanism of A3B overexpression and the A3B deletion polymorphism in human tumorigenesis remains to be determined. Moreover, ethnic differences have been observed between the A3B deletion polymorphism and their association with the occurrence of cancer. The frequency of the A3B deletion variant was shown to be 6% among individuals of European descent, 37% among individuals of Asian descent, and 57.7% among individuals of American descent (27).

The relationship between A3B gene deletion and the risk of non-small cell lung cancer in the Chinese population has not been studied yet, and the effects of interaction between A3B deletion polymorphism and environmental factors on the risk of NSCLC remain unclear. To identify the A3B deletion polymorphism relevant to NSCLC, we investigated the genotype distribution of A3B deletion in NSCLC and explored their association with NSCLC risk to provide novel biomarkers for early prevention and prognosis of NSCLC in the southern Chinese population. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/atm-21-989).

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#Methods

##Ethical statement

All subjects were supplied with written informed consent forms for both clinical epidemiological investigations and blood sample collection before enrolment. The patient or family involved in the study provided informed consent. This study was conducted in accordance with the recommendations of the Helsinki Declaration (as

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revised in 2013), and the ethics committee of Guangdong Provincial People's Hospital approved the study protocol.

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##Study subjects and data collection

This case-control study was conducted based on the population from Guangzhou, Guangdong Province. A total of 317 NSCLC cases and 334 controls were included in this study. The inclusion criteria for the cases were as follows: (I) diagnosed as NSCLC by histopathological examination; (II) they had not undergone previous systemic therapy for metastatic disease; (III) had an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1; (IV) had at least 1 measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (28, 29) The control group consisted of healthy people who came to the hospital for medical examinations, or patients without cancer or other diseases that might have been related to lung cancer. In order to obtain their demographic characteristics and information on environmental exposures, we conducted a questionnaire survey. EDTA anticoagulation tubes (3-5 mL) were used to collect peripheral blood, and the blood was stored at -80ºC. Participants who smoked less than 100 cigarettes in their lifetime were defined as non-smokers, otherwise, they were classified as smokers.

Power analysis was performed for this study by Power and Sample Size (PASS 15). The sample size for 90.1% power value is 330 persons in total, and our sample size has been a total of 651 subjects.

##Genotype analysis of the A3B deletion polymorphism

The Blood DNA Kit (OMEGA) was used to extract peripheral blood DNA according to the manufacturer's protocol, and the samples were stored at −20 C°. We then used a TaKaRa Premix Taq kit to perform the PCR. Insertion primers amplified the insertion sequence configuration and generation of the 490 bp PCR product. Deletion primers amplified the deletion sequence configuration and generation of the 700 bp PCR product. The genotype primers included insertion forward primer: TTGGTGCTGCCCCCTC, reverse primer, TAGAGACTGAGGCCCAT; deletion forward primer, TAGGTGCCACCCCGAT, reverse primer, TTGAGCATAATCTTACTCTTGTAC. The 25 μL reaction volume included 2 μL primer F and 2 μL primer R, 2 μg DNA, 12.5 μL Premix Taq, and 6.5 μL sterilized

water. The conditions of PCR were as follows: 95.0 C° for 5 min, followed by 40 cycles at 95.0 ℃for 5 min, 61.0 C° for 1 min, and 72.0 C° for 1 min, extension for 7 min at 72.0 C°, with a final step at 4.0 C°. Agarose gel electrophoresis was employed to analyze the PCR products (Figure 1).

Among the 334 controls, 152 were homozygous for the A3B insertion allele, accounting for 45.51%, while 138 (41.32%) were heterozygous, and 44 (13.17%) were homozygous for the deletion allele. This resulted in an observed minor allele frequency (MAF) of 1.989, and the genotype distribution did not deviate from the Hardy-Weinberg equilibrium (P>0.05).

##Statistical analysis

The demographic data and genotype characteristics are presented as number (percentage), and the mean ± SD. For categorical variables, the χ^2-test was performed to calculate the differences between demographic variables and genotype distribution of A3B deletion between the case group and the control group. For continuous variables with a normal distribution, an ANOVA was performed **(Table 1 and Table S1)**. Logistic regression analyses were used to calculate the odds ratios (ORs), the 95% confidence intervals (CIs), and to estimate the associations between A3B deletion and NSCLC risk. Model 1 was unadjusted. Model 2 was adjusted for age, gender, and smoking status **(Table 2)**. The Hardy-Weinberg equilibrium (HWE) of the control group was calculated by χ^2 statistics. Statistical significance was set as P<0.05, and all tests were two-sided. All of the statistical analyses were performed using STATA.

The multifactor dimensionality reduction (MDR) software was used to identify the best gene-environment interaction model of A3B deletion. The environmental factors we included in the MDR were age, gender, and smoking status **(Table 3)**.

#Results

##Population characteristics

The baseline characteristics of all included individuals are presented in **Table 1**. There were significant differences between the NSCLC group and the control group in age, gender, smoking, and genotype distribution (P<0.05). In the case group, the population over 55 years old accounted for 65.93%, compared to only 32.54% in the control group.

The case group was also 55% male, compared to 35.03% male in the control group. The probability of detecting the del/del genotype was higher in the case group than the control group (27.13% vs. 13.17%, P<0.001).

##Association between the A3B deletion genotype and NSCLC

A significant association between the A3B del/del genotype and the risk of NSCLC was identified after adjusting for confounding factors. The individuals carrying the del/del genotype were at a 2.71 times higher risk of developing NSCLC than those with the ins/ins genotype.

##Association between genotype distributions in NSCLC patients and clinical information

The clinical information of the case group is shown in **Table S1**. However, we did not identify differences between clinical information (tumor size, lymph node metastasis, clinical stage) and genotype distribution.

##The interaction between A3B deletion genotypes and environmental factors

To explore the roles of gene-environment interactions on the risk of NSCLC, generalized multifactor dimensionality reduction (GMDR) was conducted to analyze the interaction between A3B deletion genotypes and environmental factors in NSCLC. The results showed that the best interaction model of NSCLC was the 3-factor model, including A3B deletion genotype, gender, and smoking **(Table 3)**.

Compared with the reference group (ins/ins genotype, female, and non-smoking), individuals who carried the A3B ins/del genotype and were females and non-smokers had a lower risk of NSCLC. Regardless of smoking status, males had a higher risk of developing NSCLC with the ins/del genotype (1.907 and 3.375), and the risk among smokers was higher. Among individuals who carried the del/del genotype, males had approximately 7.312- and 7.769-time increased risk of developing NSCLC, and smokers had a higher risk of NSCLC**(Table 4, Figure 2)**.

#Discussion

We conducted a hospital-based case-control study to determine whether

the A3B deletion polymorphism increased NSCLC risk among the southern Chinese population. In this study, we observed that individuals carrying the del/del genotype in the A3B deletion polymorphism had a 2.71-fold higher risk of developing NSCLC than those with the ins/ins genotype. Our results are consistent with previous findings in Norway (16), suggesting that A3B deletion may be useful as a biomarker for screening NSCLC in the early stages. Using the best 3-factor interaction model, we found that the A3B deletion polymorphism interacts with smoking and gender to increase the risk of developing NSCLC. To the best of our knowledge, this is the first study to explore the correlations between the A3B deletion polymorphism with an increased risk of NSCLC among the southern Chinese population. It therefore provides an important molecular epidemiological perspective on the correlations between the A3B deletion polymorphism and NSCLC.

Recent studies have reported that the A3B deletion polymorphism is associated with an increased risk of various cancer types, including breast (11, 30, 31), colon (16), cervical (17), lung (16), prostate (16), ovarian (18), bladder (19), oral (17), and hepatocellular carcinoma (20). A3B deletion is also correlated with an increased mutational load (21). Apart from deleting the A3B coding frame, A3B deletion carriers also have the ability to generate a novel fusion transcript that is fused to the A3B 3'UTR (27). We are curious how this will affect the expression of A3B. However, the expression of A3B is not only regulated by the A3B gene, the expression of A3B is also regulated by the p53 gene. P53 mutations up-regulated the expression of A3B(15). Consequently, A3A mRNA in the fusion transcript has been shown to become more stable (10- to 20-fold higher steady-state levels of A3A), resulting in higher levels of A3A and more severe DNA damage (22). Since A3A is capable of acting as a hypermutator to break DNA, and is sensitive to inflammatory environments involving type I and II interferons, this can result in chronic inflammation which has long been associated with the onset of cancer (32). Moreover, repeated A3A-induced DNA damage is a major driving force behind iterative somatic mutations of the human genome (22). The frequency of the A3B deletion polymorphism varies across different populations, as it is rare in Africans and Europeans (0.9% and 6%), but more common in East Asians and Amerindians (36.9% and 57.7%) (27). In our southern Chinese population, 57.3% of individuals carried the A3B deletion allele. It may explain the heterogeneity in the associations between A3B deletion and cancer risk in different

ethnicities. The associations stay consistent in Asian populations (17, 33, 34), while the associations are inconsistent in Europeans (16, 18, 35).

Furthermore, we evaluated the association between the A3B deletion polymorphism and the clinical features of the 317 NSCLC patients. However, we found that the A3B deletion polymorphism was not significantly associated with lymph node metastasis number, tumor size, and clinical stage, which is consistent with a previous study in breast cancer (36). Gansmo et al. observed that lung cancer was associated with the deletion allele in Caucasian individuals under 50 years old, and the OR gradually decreased with increasing age (16). However, this observation was not reproduced in our research. We classify the age group with 55 years old as the boundary value, there is also no association between APOBEC3A/B deletion and the age at diagnosis

Recently, it has been reported that susceptibility to NSCLC could be affected by various factors, including interactions between various environmental factors (37, 38) and different polymorphisms (39). Using the best 3-factor interaction model, we observed that the A3B deletion polymorphism interacts with smoking and gender to increase the possibility of developing NSCLC. Since the A3B deletion polymorphism is a genetic factor, along with gender it cannot be altered. Hence, smoking is the only modifiable variable. As a result, by controlling smoking, how much can the risk of NSCLC based on these 3 characteristics be reduced? Smoking among males with the A3B del/del genotype was associated with a 5.9% increased risk of NSCLC ($OR_{del/del\ +smoking\ male}$: 7.77 vs. $OR_{del/del\ +non\text{-}smoking\ male}$: 7.31) compared to non-smoking females with the ins/ins genotype. Furthermore, males with the A3B ins/del genotype had significantly increased NSCLC risk by 43.5% ($OR_{del/del\ +smoking\ male}$: 3.38 vs. $OR_{del/del\ +non\text{-}smoking\ male}$: 1.91), compared to non-smoking females with the ins/ins genotype. This outcome is consistent with the analysis of the clinical data between healthy control individuals and NSCLC patients, which showed that smoking is related to lung tumorigenesis (40). However, due to the limited female smoker samples, the power to precisely predict the cancer risk of smoking females with the A3B deletion genotype is also limited.

There are some limitations in our study. First, the sample in the study was not large, which might have restricted the ability to explore weaker associations among NSCLC, environmental factors, and the A3B deletion polymorphism. What's more, our sample

was selected from the southern population in China and cannot represent the whole population. Second, the clinical characteristics of the included individuals collected were not very comprehensive. Several prognostic characteristics were not collected, such as survival time and tumor recurrence status. In addition, we should have also included more meaningful environmental factors in our analysis, such as diet and passive smoke exposure. Therefore, further large and well-designed investigations are needed in the future to support our findings.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-21-989). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The patient or family involved in the study provided informed consent. This study was conducted in accordance with the recommendations of the Helsinki Declaration (as revised in 2013), and the ethics committee of Guangdong Provincial People's Hospital approved the study protocol.

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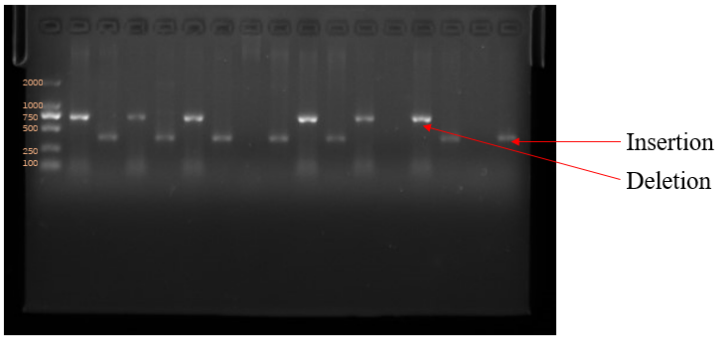

Figure 1 The results of the agarose gel electrophoresis.

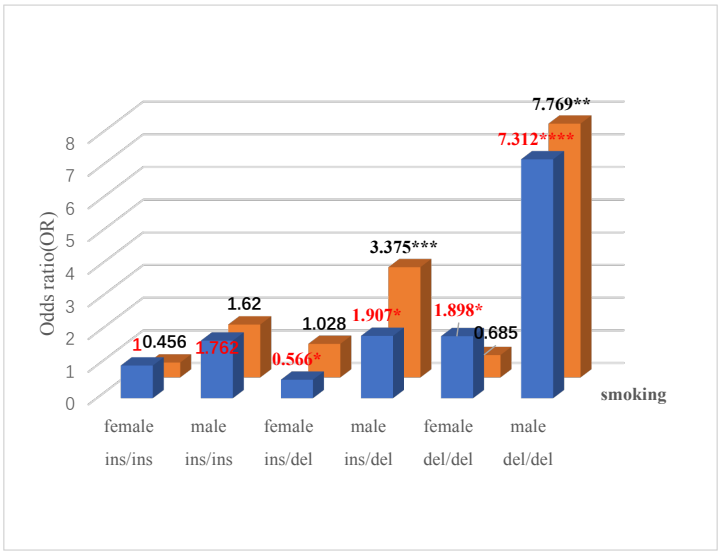

Figure 2 Risk factor analysis of gene-environment interactions in non-small cell lung cancer (NSCLC). Model: APOBEC3B deletion genotypes, gender, and smoking. The reference group was the interaction of ins/ins genotype for APOBEC3B, gender, and smoking status. The odds ratio (OR) value is shown in the figure. *P<0.05 and **P<0.01, ***P<0.001, ****P<0.000.

Table 1 The baseline population characteristics of the control group and NSCLC patients

Characteristic	Case	Control	χ^2	P
Age			72.694	<0.001
<55	108 (34.07)	226 (67.46)		
⩾55	209 (65.93)	109 (32.54)		
Gender			26.763	<0.001
Male	175 (55.21)	117 (35.03)		
Female	142 (44.79)	217 (64.97)		
Smoking			7.923	0.005
Yes	236 (74.45)	260 (83.60)		
No	81 (25.55)	51 (16.40)		
Genotype			20.052	<0.001
Wildtype (II)	126 (39.75)	152 (45.51)		

Hetero (ID)	105 (33.12)	138 (41.32)
Deletion (DD)	86 (27.13)	44 (13.17)

NSCLC, non-small cell lung cancer.

Table 2 Association between the A3B deletion polymorphism and NSCLC

Genotype	Case		Control		OR	P value	95% CI
	N	Percent (%)	N	Percent (%)			
Model 1							
Wildtype (II)	126	39.75	152	45.51	Reference		
Hetero (ID)	105	33.12	138	41.32	0.92	0.628	0.65–1.30
Deletion (DD)	86	27.13	44	13.17	2.36	<0.001	1.53–3.64
Model 2							
Wildtype (II)	126	39.75	141	45.48	Reference		
Hetero (ID)	105	33.12	132	42.58	0.87	0.466	0.59–1.27
Deletion (DD)	86	27.13	37	11.94	2.71	<0.001	1.67–4.42

Model 1: unadjusted model; Model 2: adjusted for gender, age, smoking. NSCLC, non-small cell lung cancer; OR, odds ratio; CI, confidence interval.

Table 3 The gene-environment interaction model for NSCLC using MDR analysis

Model	TBA	CVC	P value
Genotype	0.6004	10/10	0.001
Genotype*smoking	0.6318	10/10	0.001
Genotype*gender*smoking	0.6415	10/10	0.001

*, gene-environment interaction model. NSCLC, non-small cell lung cancer; MDR, multifactor dimensionality reduction.

Table 4 Risk factor analysis of NSCLC based on 3 factors: genotype, gender, and smoking

APOBEC3B	Gender	Smoking status	Case	Control	OR (95% CI)	*P* value
ins/ins	Female	−	62	85	reference	
ins/ins	Female	+	2	6	0.456 (0.089–2.340)	0.347

ins/ins	Male	−	32	23	1.762 (0.975–3.188)	0.061
ins/ins	Male	+	26	22	1.620 (0.841–3.120)	0.149
ins/del	Female	−	38	92	0.566 (0.343–0.933)	0.026*
ins/del	Female	+	3	4	1.028 (0.222–4.760)	0.972
ins/del	Male	−	32	23	1.907 (1.018–3.574)	0.044*
ins/del	Male	+	32	14	3.375 (1.638–6.954)	0.001*
del/del	Female	−	36	26	1.898 (1.040–3.463)	0.037*
del/del	Female	+	1	2	0.685 (0.061–7.729)	0.760
del/del	Male	−	32	6	7.312 (2.881–27.671)	0.000*
del/del	Male	+	17	3	7.769 (2.034–28.510)	0.002*

*, P<0.05. NSCLC, non-small cell lung cancer; OR, odds ratio.

Table S1. Correlation between APOBEC3B and clinical pathology parameters in NSCLC.

Variable	N	genotype			x^2/F	*P*-value
		Wildtype	Hetero	Deletion		
age					5.591	0.061
<55	108	51(40.48)	27(25.71)	30(34.88)		
≥55	209	75(59.52)	78(74.29)	56(65.12)		
gender					3.345	0.188
male	175	62(49.21)	64(60.95)	49(56.98)		
female	142	64(50.79)	41(39.05)	37(43.02)		
smoking	81	28(22.22)	35(33.33)	18(20.93)	5.042	0.08
drinking	19	10(8.85)	7(7.69)	2(2.56)	3.096	0.213
tumor	276	2.04±1.59	1.89±1.26	2.14±1.51	0.610	0.543
Lymph node metastasis	41	16(14.41)	11(12.50)	14(18.18)	1.077	0.584
Clinical stage					1.129	0.569
< II	221	89(73.55)	75(76.53)	57(69.51)		
>II	80	32(26.45)	23(23.47)	25(30.49)		
diabetes	25	15(13.27)	4(4.4)	6(7.69)	5.102	0.078
hypertension	61	28(24.78)	13(14.29)	20(25.64)	4.297	0.117

obesity	18	8(23.53)	3(10.34)	7(26.92)	2.708	0.258
education					4.552	0.602
Illiteracy	15	8(7.55)	5(5.68)	2(2.90)		
Low	116	43(40.57)	44(50.00)	29(42.03)		
Mid	61	28(26.42)	16(18.18)	17(24.64)		
High	71	27(25.47)	23(26.14)	21(30.43)		

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ICMJE DISCLOSURE FORM

Date:_21/3/1_____
Your Name:_ Shan Liu_____
Manuscript Title:_ APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population_____
Manuscript number (if known):_____

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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The author's relationships/activities/interests should be defined broadly. For example, if your manuscript pertains to the epidemiology of hypertension, you should declare all relationships with manufacturers of antihypertensive medication, even if that medication is not mentioned in the manuscript.

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Time frame: Since the initial planning of the work			
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Time frame: past 36 months			
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6	Payment for expert testimony	____None	
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13	Other financial or non-financial interests	____None	

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Date:_21/3/1_____
Your Name:_ Xin Xie_____
Manuscript Title:_ APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population_____
Manuscript number (if known):_____

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Date:_21/3/1_____
Your Name:_ Yuting Ying _____
Manuscript Title:_ APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population_____
Manuscript number (if known):_____

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Date:_21/3/1_____
Your Name:_ Guibin Qiao _____
Manuscript Title:_ APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population_____
Manuscript number (if known):_____

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Date:_21/3/1_____
Your Name:_ Chunxia Jing _____
Manuscript Title:_ APOBEC3B deletion polymorphism and lung cancer risk in the southern Chinese population_____
Manuscript number (if known):_____

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