RESEARCH ARTICLE

P53 Arg72Pro and MDM2 SNP309 Polymorphisms Cooperate to Increase Lung Adenocarcinoma Risk in Chinese Female Non-smokers: A Case Control Study

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Abstract

Background: Cell cycle deregulation is a major component of carcinogenesis. The *p53* tumor suppressor gene plays an important role in regulating cell cycle arrest, and mouse double minute 2 (MDM2) is a key regulator of *p53* activity and degradation. Abnormal expression of *p53* and MDM2 occurs in various cancers including lung cancer. Methods: We investigated the distribution of the *p53* Arg72Pro (rs1042522) and MDM2 SNP309 (rs2279744) genotypes in patients and healthy control subjects to assess whether these single nucleotide polymorphisms (SNPs) are associated with an increased risk of lung adenocarcinomas in Chinese female non-smokers. Genotypes of 764 patients and 983 healthy controls were determined using the TaqMan SNP genotyping assay. Results: The *p53* Pro/Pro genotype (adjusted OR = 1.55, 95% CI = 1.17–2.06) significantly correlated with an increased risk of lung adenocarcinoma, compared with the Arg/Arg genotype. An increased risk was also noted for MDM2 GG genotype (adjusted OR = 1.68, 95% CI = 1.27–2.21) compared with the TT genotype. Combined *p53* Pro/Pro and MDM2 GG genotypes (adjusted OR = 2.66, 95% CI = 1.54–4.60) had a supermultiplicative interaction with respect to lung adenocarcinoma risk. We also found that cooking oil fumes, fuel smoke, and passive smoking may increase the risk of lung adenocarcinomas in Chinese female non-smokers who carry *p53* or MDM2 mutant alleles. Conclusions: *P53* Arg72Pro and MDM2 SNP309 polymorphisms, either alone or in combination, are associated with an increased lung adenocarcinoma risk in Chinese female non-smokers.

Keywords: ***P53*** - MDM2 - polymorphism - lung cancer - susceptibility

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Introduction

Lung cancer is one of the leading causes of cancer-related deaths in the world (Jemal et al.,2011). Cigarette smoking is responsible for about 80% of lung cancers; however, only 20% of smokers develop lung cancer (Smyth, 1996; Pesch et al., 2012). Unlike smokers, who commonly develop squamous cell carcinomas, non-smokers (especially females) are more likely to develop adenocarcinomas (Zang et al., 1996), suggesting that genetic susceptibility and environmental factors may differentially affect lung cancer development. Single nucleotide polymorphisms (SNPs) in several genes involved in carcinogen metabolism, DNA damage repair, cell cycle control, and apoptosis are reported to be associated with lung cancer (Wu et al., 2002; Li et al., 2008; Yin et al., 2009). However, lung cancer development is a multistage process involving both genetic mutations and environmental risk factors. In Chinese women, lung cancer mortality is much higher than expected from the low prevalence of smoking (<4%) in this population (Jemal et al., 2011), indicating that Chinese women may be genetically susceptible to lung cancer development. Therefore, it is important to identify genes and gene–gene interactions that contribute to lung adenocarcinoma risk in Chinese women.

The *p53* tumor suppressor protein regulates a number of cellular functions such as cell cycle arrest, gene transcription, and apoptosis in response to DNA damage (Kastan et al., 1991; Dulic et al., 1994). In as many as 50% of human cancers, the *p53* gene is inactivated by mutation or deletion (Jin et al., 2001; Lain et al., 2003). The human homolog of mouse double minute 2 (MDM2) negatively regulates *p53* by a number of different mechanisms: it inhibits *p53* transcriptional activity by directly binding to transactivation domain of the *p53* gene (Momand et al., 1992) and directly promotes *p53* protein degradation through its E3 ubiquitin ligase activity (Haupt et al., 1997). MDM2 overexpression has been reported in non-small cell lung carcinoma, where it may substitute for *p53* protein

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inactivation (Eymin et al., 2002). By inhibiting MDM2 or blocking the *p53*–MDM2 interaction, tumor cells can reactivate *p53* and thereby inhibit tumor growth (Chene, 2003). Thus, *p53* and MDM2 play important roles in the development of lung cancer.

The *p53* gene harbors a G→C polymorphism in codon 72 that results in the replacement of arginine with proline (Arg72Pro, rs1042522) (Ara et al., 1990). The *p53* codon 72 proline allele is less efficient than arginine allele at inducing apoptosis (Dumont et al., 2003). Genetic polymorphisms also exist in the MDM2 gene. For example, Bond et al. (Bond et al., 2004) described a common polymorphism located at position 309 in the first intron of MDM2 gene (SNP309, rs2279744) that leads to increased MDM2 expression and an earlier age of onset of some types of cancer, suggesting that this SNP may play an important role in cancer development. A number of studies have reported an association between *p53* Arg72Pro and MDM2 SNP309 polymorphisms and lung cancer (Jassem et al., 2005; Hu et al., 2006; Lind et al., 2006; Zhang et al., 2006; Caceres et al., 2009); however, results in different populations are contradictory and none of the studies considered the effects of environmental factors other than smoking.

In this case control study, we investigated the distribution of *p53* Arg72Pro and MDM2 SNP309 genotypes in Chinese female non-smokers to determine whether these SNPs are associated with an increased risk of lung adenocarcinoma. We also examined gene–gene and gene–environment interactions between these SNPs and potential environmental risk factors.

Materials and Methods

Ethics statement

This investigation was approved by the Institutional Review Board of China Medical University. Written informed consent was obtained from each participant.

Study subjects

Part of the patients used in this hospital-based case control study was described previously (Li et al., 2008; Yin et al., 2009). We included 764 lung adenocarcinoma patients with histologically confirmed diagnoses between January 2002 and December 2012. During the same period, 983 healthy controls with no evidence of lung or other cancer were recruited from a medical examination center in the same hospital. Participants were unrelated ethnic Han Chinese women. Face-to-face interviews of patients and healthy control subjects were done by trained interviewers and demographic data, including family history of cancer, and information on exposure to environmental lung cancer risk factors, including cooking oil fumes, fuel smoke, occupational exposure, and passive smoking, were collected. Estimates of the exposure of each participant to environmental factors were reported previously (Li et al., 2008; Yin et al., 2009).

Genotyping

DNA was extracted from 1 mL samples of whole blood using standard phenol–chloroform methods. Genotyping was performed on an Applied Biosystems 7500 FAST Real-Time PCR System (Foster City, CA, USA) using a TaqMan SNP genotyping assay (Affymetrix Inc., Cleveland, Ohio, USA). Each reaction (10 μL) contained 5 μL TaqMan Genotyping master mix, 0.5 μL primers and probes (Applied Biosystems), 2.5 μL water and 2 μL DNA (15–25 ng/μL). Thermal cycling conditions were 95°C for 10 min, followed by 47 cycles of 92°C for 30 sec and 62°C for 1 min. Duplicates of 10% of the samples were re-tested for quality control purposes.

Statistical analysis

The chi-squared test and Student's t-test were separately used to examine differences in categorical and continuous variables between patients and controls. Hardy–Weinberg equilibrium was tested using a Pearson chi-squared test. The odds ratio (OR) and its 95% confidence interval (95% CI) were obtained by logistic regression methodology to determine correlations between the two polymorphisms, exposure to environmental risk factors, and the incidence of lung adenocarcinoma. We also investigated gene–gene and gene–environment interactions using logistic regression analysis. All analyses were performed using SPSS 13.0 software (SPSS, Inc. Chicago, IL, USA), and a $P < 0.05$ was considered to be statistically significant.

Results

A total of 852 lung cancer patient (including 77 duplicates) and 1084 healthy control (including 99

Table 1. Demographics of Lung Cancer Patients and Healthy Controls

Variable	No. of patients (%)	No. of controls (%)	OR(95% CI)	*P* value
Total no.	764	983		
Age (years)	56.47 ± 11.28	56.04 ± 12.11		0.437
Income (Yuan/month)	567.32 ± 367.27	539.84 ± 370.73		0.124
Education				0.570
None	44 (5.8)	49 (5.0)		
Elementary school	322 (42.1)	412 (41.9)		
Junior school	272 (35.6)	337 (34.3)		
Senior school and upwards	126 (16.5)	185 (18.8)		
Fuel smoke exposure	226 (29.6)	263 (26.8)	1.15 (0.93–1.42)	0.192
Cooking oil fume exposure	246 (32.2)	245 (24.9)	1.43 (1.16–1.76)	0.001
Family history of cancer	106 (13.9)	102 (10.4)	1.39 (1.04–1.86)	0.025
Passive smoking	551 (72.1)	684 (69.6)	1.13 (0.92–1.39)	0.248

Table 2. Genotype Frequencies of *p53* Arg72Pro and MDM2 SNP309 Polymorphisms among Patients and Controls and Their Association with Lung Adenocarcinoma Risk

Polymorphism	No. of patients (%)	No. of controls (%)	OR (95% CI)	*P* value
P53 Arg72Pro				
Arg/Arg	154 (20.2)	246 (25.0)	Reference	
Arg/Pro	413 (54.1)	522 (53.1)	1.30 (1.02–1.65)	0.035
Pro/Pro	197 (25.8)	215 (21.9)	1.55 (1.17–2.06)	0.002
Arg/Pro + Pro/Pro	610 (79.8)	737 (75.0)	1.37 (1.09–1.73)	0.008
MDM2 SNP309				
TT	202 (26.4)	335 (34.1)	Reference	
TG	391 (51.2)	472 (48.0)	1.42 (1.13–1.77)	0.002
GG	171 (22.4)	176 (17.9)	1.68 (1.27–2.21)	<0.001
TG + GG	562 (73.6)	548 (65.9)	1.49 (1.21–1.84)	<0.001

OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, fuel smoke exposure, and cooking oil fume exposure

Table 3. Interaction of *p53* Arg72Pro and MDM2 SNP309 Polymorphisms on Lung Adenocarcinoma Risk

P53 Arg 72Pro	MDM2 SNP309	No. of patients (%)	No. of controls (%)	OR (95% CI)[a]	*P* value
Arg/Arg	TT	52 (6.8)	98 (10.0)	Reference	
Arg/Arg	TG	79 (10.3)	118 (12.0)	1.34 (0.86–2.09)	0.200
Arg/Arg	GG	23 (3.0)	30 (3.1)	1.51 (0.79–2.89)	0.209
Arg/Pro	TT	100 (13.1)	168 (17.1)	1.16 (0.76–1.77)	0.497
Arg/Pro	TG	215 (281)	246 (25.0)	1.78 (1.20–2.62)	0.004
Arg/Pro	GG	98 (12.8)	108 (11.0)	1.88 (1.21–2.93)	0.005
Pro/Pro	TT	50 (6.5)	69 (7.0)	1.52 (0.92–2.51)	0.104
Pro/Pro	TG	97 (12.7)	108 (11.0)	1.84 (1.19–2.86)	0.006
Pro/Pro	GG	50 (6.5)	38 (3.8)	2.66 (1.54–4.60)	<0.001

[a] OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, fuel smoke exposure, and cooking oil fume exposure

duplicates) samples were genotyped. Genotyping was unsuccessful for 12 patient and two healthy control samples. However, all duplicate samples were concordant. After discarding duplicate samples, the study included 764 patients and 983 controls. The demographic characteristics of patients and controls, and their incidence of exposure to environmental lung cancer risk factors, are shown in Table 1. The mean ages of patients and healthy controls were almost identical (56.47 ± 11.28 and 56.04 ± 12.11 years). In addition, levels of education, as well as fuel smoke exposure and passive smoking, were similar between patients and controls. However, patients were more likely to have been exposed to cooking oil fumes ($P = 0.001$) and to have a family history of cancer ($P = 0.025$).

Distributions of the *p53* Arg72Pro and MDM2 SNP309 polymorphisms in patients and control subjects are shown in Table 2. The allele frequencies of *p53* Pro and MDM2 G were 0.485 and 0.419, respectively, in healthy controls. The genotype frequencies for *p53* Arg72Pro and MDM2 SNP309 polymorphisms in control subjects conformed to the Hardy–Weinberg equilibrium ($P = 0.055$ and $P = 0.694$, respectively). Multivariate logistic regression analysis determined the ORs for Arg/Pro and Pro/Pro genotypes to be 1.30 (95% CI, 1.02–1.65) and 1.55 (95% CI, 1.17–2.06), respectively, compared with *p53* Arg/Arg genotype, after adjusting for age, family history of cancer, and passive smoking, fuel smoke exposure, and

Table 4. Interaction of *p53* Arg72Pro and Exposure to Environmental Factors on Lung Adenocarcinoma Risk

Environmental exposure	*p53* Arg 72Pro	No. of patients (%)	No. of controls (%)	OR (95% CI)	*P* value
Cooking oil fumes					
-	Arg/Arg	99 (13.0)	181 (18.4)	Reference	
-	Arg/Pro	277 (36.3)	387 (39.4)	1.34 (1.00–1.79)[a]	0.048
-	Pro/Pro	142 (18.6)	170 (17.1)	1.56 (1.12–2.18)[a]	0.009
+	Arg/Arg	55 (7.2)	65 (6.6)	1.53 (0.99–2.37)[a]	0.057
+	Arg/Pro	136 (17.8)	135 (13.9)	1.86 (1.32–2.62)[a]	<0.001
+	Pro/Pro	55 (7.2)	45 (4.6)	2.27 (1.43–3.62)[a]	0.001
Fuel smoke					
-	Arg/Arg	98 (12.8)	171 (17.4)	Reference	
-	Arg/Pro	305 (39.9)	400 (40.7)	1.34 (1.00–1.80)[b]	0.049
-	Pro/Pro	135 (17.7)	149 (15.2)	1.63 (1.16–2.30)[b]	0.005
+	Arg/Arg	56 (7.3)	75 (7.6)	1.15 (0.75–1.78)[b]	0.523
+	Arg/Pro	108 (14.1)	122 (12.4)	1.41 (0.97–2.04)[b]	0.069
+	Pro/Pro	62 (8.1)	66 (6.7)	1.56 (1.01–2.41)[b]	0.043
Passive smoking					
-	Arg/Arg	56 (7.3)	83 (8.4)	Reference	
-	Arg/Pro	107 (14.0)	150 (15.3)	1.16 (0.76–1.78)[c]	0.494
-	Pro/Pro	50 (6.5)	64 (6.5)	1.25 (0.75–2.07)[c]	0.393
+	Arg/Arg	98 (12.8)	163 (16.6)	1.02 (0.65–1.58)[c]	0.948
+	Arg/Pro	306 (40.1)	374 (38.0)	1.40 (0.95–2.07)[c]	0.091
+	Pro/Pro	147 (19.2)	149 (15.2)	1.71 (1.11–2.63)[c]	0.014

[a]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and fuel smoke exposure; [b]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and cooking oil fume exposure; [c]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, fuel smoke exposure, and cooking oil fume exposure

Table 5. Interaction of MDM2 SNP309 and Exposure to Environmental Factors on Lung Adenocarcinoma Risk

Environmental exposure	MDM2 SNP309	No. of patients(%)	No. of controls(%)	OR (95% CI)[a]	*P* value
Cooking oil fumes					
-	TT	117 (15.3)	252 (25.6)	Reference	
-	TG	276 (36.1)	353 (35.9)	1.69 (1.29–2.21)[a]	<0.001
-	GG	125 (16.4)	133 (13.5)	2.02 (1.45–2.81)[a]	<0.001
+	TT	85 (11.1)	83 (8.4)	2.12 (1.46–3.10)[a]	<0.001
+	TG	115 (15.1)	119 (12.1)	2.09 (1.49–2.93)[a]	<0.001
+	GG	46 (6.0)	43 (4.4)	2.31 (1.44–3.71)[a]	0.001
Fuel smoke					
-	TT	142 (18.6)	250 (25.4)	Reference	
-	TG	280 (36.6)	328 (33.4)	1.53 (1.17–1.98)[b]	0.002
-	GG	116 (15.2)	142 (14.4)	1.46 (1.06–2.02)[b]	0.022
+	TT	60 (7.9)	85 (8.6)	1.06 (0.71–1.59)[b]	0.761
+	TG	111 (14.5)	144 (14.6)	1.27 (0.91–1.76)[b]	0.155
+	GG	55 (7.2)	34 (3.5)	2.67 (1.65–4.33)[b]	<0.001
Passive smoking					
-	TT	64 (8.4)	105 (10.7)	Reference	
-	TG	108 (14.1)	158 (16.1)	1.16 (0.78–1.73)[c]	0.469
-	GG	41 (5.4)	36 (3.7)	2.03 (1.17–3.53)[c]	0.012
+	TT	138 (18.1)	230 (23.4)	1.07 (0.72–1.59)[c]	0.730
+	TG	283 (37.0)	314 (31.9)	1.67 (1.15–2.41)[c]	0.007
+	GG	130 (17.0)	140 (14.2)	1.72 (1.15–2.59)[c]	0.009

[a]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and fuel smoke exposure; [b]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and cooking oil fume exposure; [c]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, fuel smoke exposure, and cooking oil fume exposure

cooking oil fume exposure. Participants carrying the Arg/Pro or Pro/Pro genotype had an elevated adjusted OR of 1.37 (95% CI, 1.09–1.73), compared with Arg/Arg genotype carriers. Similar results were obtained for MDM2 SNP309 genotypes: adjusted ORs for the TG and GG genotypes were 1.42 (95% CI, 1.13–1.77) and 1.68 (95% CI, 1.27–2.21), respectively, compared with the TT genotype. In addition, individuals with a TG or GG genotype had a 1.49-fold increased risk of lung cancer

Table 6. Interaction of *p53* Arg72Pro, MDM2 SNP309 and Exposure to Environmental Factors on Lung Adenocarcinoma Risk

Environmental exposure	No. of mutation alleles	No. of patients(%)	No. of controls(%)	OR (95% CI)	*P* value
Cooking oil fumes					
-	0	26 (3.4)	71 (7.2)	Reference	
-	1–2	314 (41.1)	468 (47.6)	1.85 (1.15–2.97)[a]	0.011
-	3–4	178 (23.3)	199 (20.2)	2.48 (1.51–4.07)[a]	<0.001
+	0	26 (3.4)	27 (2.7)	2.50 (1.23–5.07)[a]	0.011
+	1–2	153 (20.0)	163 (16.6)	2.58 (1.56–4.26)[a]	<0.001
+	3–4	67 (8.8)	55 (5.6)	3.35 (1.88–5.96)[a]	<0.001
Fuel smoke					
-	0	26 (3.4)	71 (7.2)	Reference	
-	1–2	349 (45.7)	458 (46.6)	2.09 (1.30–3.37)[b]	0.002
-	3–4	163 (21.3)	191 (19.4)	2.40 (1.45–3.95)[b]	0.001
+	0	26 (3.4)	27 (2.7)	2.16 (1.06–4.42)[b]	0.035
+	1–2	118 (15.4)	173 (17.6)	1.71 (1.02–2.87)[b]	0.043
+	3–4	82 (10.7)	63 (6.4)	3.40 (1.93–6.00)[b]	<0.001
Passive smoking					
-	0	20 (2.6)	39 (4.0)	Reference	
-	1–2	130 (17.0)	189 (19.2)	1.55 (0.85–2.80)[c]	0.152
-	3–4	63 (8.2)	71 (7.2)	2.05 (1.07–3.92)[c]	0.030
+	0	32 (4.2)	59 (6.0)	1.26 (0.62–2.57)[c]	0.947
+	1–2	337 (44.1)	442 (45.0)	1.82 (1.02–3.25)[c]	0.091
+	3–4	182 (23.8)	183 (18.6)	2.43 (1.33–4.44)[c]	0.004

[a]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and fuel smoke exposure; [b]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, passive smoking, and cooking oil fume exposure; [c]OR (95% CI) were calculated by logistic regression, adjusted for age, family history of cancer, fuel smoke exposure, and cooking oil fume exposure

development compared with TT carriers.

We next examined whether a correlation existed between the *p53* and MDM2 polymorphisms (Table 3). Using *p53* Arg/Arg and MDM2 TT genotype carriers as a reference, we found that individuals carrying the *p53* Pro/Pro and MDM2 GG genotype had much higher risk of lung adenocarcinoma (2.66) than either *p53* Pro/Pro and MDM2 TT genotype carriers (1.52) or *p53* Arg/Arg and MDM2 GG genotype carriers (1.51). These results indicate that a supermultiplicative interaction exists between the *p53* Pro/Pro and MDM2 GG genotypes in lung adenocarcinoma development (Brennan, 2002).

Gene–environment interactions were also investigated (data shown in Tables 4 and 5). We detected a higher risk of developing lung adenocarcinoma in *p53* Pro/Pro genotype carriers exposed to cooking oil fumes or passive smoking: OR = 2.27 and 95% CI = 1.43–3.62 for *p53* Pro/Pro genotype carriers exposed to cooking oil fumes; OR = 1.71 and 95% CI = 1.11–2.63 for *p53* Pro/Pro genotype carriers exposed to passive smoking. These data suggest that a multiplicative interaction exists between these pairs of genetic and environmental factors. Relative to TT genotype carriers without cooking oil fume exposure, the OR (2.31) for GG genotype carriers with prior exposure to cooking oil fumes is larger than the OR (2.12) for MDM2 TT carriers with cooking oil fume exposure or the OR (2.01) for MDM2 GG genotype carriers without cooking oil fume exposure, but less than expected. These results suggest that antagonism exists between MDM2 SNP309 genotypes and cooking oil fume exposure. Similar results were obtained when MDM2 SNP309 and passive smoking were examined.

Results of the combined analysis of polymorphisms and environmental factor exposure are shown in Table 6. Subjects were divided into three groups according to the number of mutant alleles (0, 1–2, 3–4) they carry. Compared with individuals without mutant alleles and cooking oil fume exposure, ORs for individuals carrying 3–4 mutant alleles (without smoke/fume exposure) or those exposed to cooking oil fumes (without mutations) were 2.48 and 2.50, respectively. However, for individuals carrying 3–4 mutant alleles and exposed to cooking oil fumes, the OR increased to 3.35. Similar results were obtained for subjects carrying 3–4 mutant alleles who were previously exposed to either fuel smoke or passive smoking.

Discussion

In this case control study, we examined whether (1) genetic polymorphisms in *p53* and MDM2 and (2) individual environmental factors including cooking fume exposure, fuel smoke exposure, and passive smoking, either alone or in combination, are associated with an increased lung adenocarcinoma risk. We found a statistically significant association between *p53* Arg72Pro and MDM2 SNP309 polymorphisms and lung adenocarcinoma risk. Compared with the *p53* Arg/Arg genotype, the Pro/Pro genotype correlated with a 1.55-fold increased risk of lung adenocarcinoma; in addition, there was a 1.68-fold increased risk for carriers of the MDM2 GG genotype compared with the MDM2 TT genotype. We also observed a supermultiplication interaction between *p53* Arg72Pro and MDM2 SNP309 polymorphisms. Cooking fume exposure, fuel smoke exposure, and passive smoking all modified the relationship between both polymorphisms and the development of lung adenocarcinoma. A two-sided test with $\alpha = 0.05$ had >80% power to detect an OR of 1.55 for the *p53* Pro/Pro genotype and an OR of 1.68 for the MDM2 GG genotype in Chinese female non-smokers.

Our observations are in line with those of several other clinical and biological studies. The allele frequencies of *p53* Pro and MDM2 G were 0.485 and 0.419, respectively, among healthy control subjects in this study; these values are similar to those of other studies on Chinese populations (0.38–0.52 and 0.42–0.52, respectively) (Hu et al., 2006; Zhang et al., 2006; Chua et al., 2010; Liu et al., 2013). Li et al. (Li et al., 2009) reported the OR for Asians carrying Pro/Pro genotype to be 1.395 (95% CI = 1.206–1.613), which is much higher than that of Caucasians. Zhang et al. (2006) found that smoking modified the association between the *p53* Arg72Pro polymorphism and lung cancer; in contrast, both Piao et al. (2011) and Sakiyama et al. (2005) found that it did not. To remove the possible confounding effects of cigarette smoking, all participants in our study were female non-smokers. Consistent with previous studies (Sakiyama et al., 2005; Piao et al., 2011), we found the *p53* Arg72Pro polymorphism to be associated with increased lung adenocarcinoma risk. This result is supported by reports that the Pro allele of the *p53* Arg72Pro polymorphism, which is less efficient at inducing apoptosis, reduces the tumor suppression function of *p53* and increases cancer risk (Thomas et al., 1999). MDM2 is a key regulator of *p53*: even a modest change in MDM2 protein levels can

affect cancer development by influencing the *p53* pathway (Bond et al., 2005). The MDM2 SNP309 functional SNP is located in the promoter region of MDM2; the G allele is associated with increased MDM2 expression, resulting in *p53* inhibition (Bond et al., 2004). We found G allele carriers to have a higher risk of lung cancer, consistent with results from one Chinese study (Zhang et al., 2006) and a recent meta-analysis (Zhuo et al., 2012). However, another study showed that this MDM2 polymorphism may not be associated with lung cancer in a Chinese population (Hu et al., 2006). These contradictory data are likely to be caused by differences in the lifestyle and geographical location of the participants because our results indicate that environmental factors can cooperate with such polymorphisms to influence lung cancer development.

Lung cancer is a complex multifactorial, polygenic disease, and therefore a single SNP or environmental factor may only have a modest effect on its development. Thus, investigating multiple biologically relevant SNPs and environmental factors may be a more accurate way of evaluating lung cancer risk. *p53* and MDM2 form a negative feedback loop that plays a central role in the DNA damage response (Momand et al., 1992). Cooking oil fume exposure, fuel smoke exposure, and passive smoking all induce DNA damage and may consequently increase lung cancer risk (Tokiwa et al., 1994; Mooney et al., 1995; Zhang et al.,2002). Therefore, we analyzed the combined effects of *p53* and MDM2 polymorphisms and environmental factor exposure. We found that exposure to cooking oil fumes or fuel smoke, as well as passive smoking, increases the risk of lung adenocarcinoma in female non-smokers who carry *p53* or MDM2 mutant alleles, indicating that both MDM2 and *p53* polymorphisms interact with all three environmental factors.

Our analysis of cooking oil fume exposure or passive smoking combined with MDM2 SNP309 revealed an interesting phenomenon: ORs for individuals carrying thee GG genotype and exposed to cooking oil fumes or passive smoking were lower than expected. This phenomenon may be linked to (1) previous reports that the MDM2 GG genotype leads to high MDM2 protein expression (Bond et al.,2004) and (2) the ability of MDM2 to negatively regulate estrogen receptor (ER) expression (Duong et al., 2007) because the estrogen signaling pathway is thought to be associated with an increased risk of developing lung cancer, especially adenocarcinoma (Karlsson et al., 2012). In addition, polycyclic aromatic hydrocarbons, which are important component of both cooking oil fumes and cigarette smoke, are reported to decrease estrogen levels (Siegfried, 2010). Thus, it is possible that a reduction in both ER expression and estrogen levels leads to a decreased lung adenocarcinoma risk in female non-smokers.

In conclusion, we have shown that *p53* Arg72Pro and MDM2 SNP309, either alone or combination, are associated with an increased lung adenocarcinoma risk in Chinese female non-smokers. Moreover, cooking oil fumes, fuel smoke, and passive smoking may increase the risk of lung adenocarcinoma in Chinese female non-smokers who carry *p53* or MDM2 mutant alleles.

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