P53 genetic polymorphisms, interactions with lifestyle factors and lung cancer risk: a case control study in a Chinese population

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Abstract

Background: A pathway-based genotyping analysis suggested rs2078486 was a novel p53 SNP, but very few studies replicate this association. P53 rs1042522 is the most commonly studied SNP, but very few studies examined its potential interaction with environmental factors in relation to lung cancer risk. This study aims to examine associations between two p53 single-nucleotide polymorphisms (SNPs) (rs2078486, rs1042522), their potential interaction with environmental factors and risk of lung cancer.

Methods: A case-control study was conducted in Taiyuan, China. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs). Multiplicative and additive interactions between p53 SNPs and lifestyle factors were evaluated.

Results: Variant p53 rs2078486 SNP was significantly associated with elevated lung cancer risk among smokers (OR: 1.70, 95% CI: 1.08 - 2.67) and individuals with high indoor air pollution exposure (OR: 1.51, 95% CI: 1.00-2.30). Significant or borderline significant multiplicative and additive interactions were found between p53 rs2078486 polymorphism with smoking and indoor air pollution exposure. The variant genotype of p53 SNP rs1042522 significantly increased lung cancer risk in the total population (OR: 1.57, 95% CI: 1.11-2.21), but there was no evidence of heterogeneity among individuals with different lifestyle factors.

Conclusions: This study confirmed that p53 rs2078486 SNP is potentially a novel p53 SNP that may affect lung cancer risk. Our study also suggested potential synergetic effects of p53 rs2078486 SNP with smoking and indoor air pollution exposure on lung cancer risk.

Keywords
Lung cancer, p53, single-nucleotide polymorphism, Chinese population

**Background**

Lung cancer is one of the most common cancers and is a leading cause of cancer death in China. It was estimated that by year 2025, more than one million Chinese will be diagnosed with lung cancer per year [1]. Lung cancer mortality increased 465% during the past 30 years and now is the leading cancer death cause in China [2]. Smoking is regarded as the most important risk factor for lung cancer, and indoor air pollution from cooking and heating is another potential risk factor in Chinese population [3]. However, approximately one in ten lifetime smokers develop lung cancer, which implies a possible role for genetic susceptibility in the development of lung cancer [4].

The p53 tumor suppressor gene plays a critical role in modulating transcription of genes that govern the major defenses against tumor growth, including cell cycle arrest, apoptosis, maintenance of genetic integrity, inhibition of angiogenesis and cellular senescence [5]. The p53 gene harbors high-frequency, functional single-nucleotide polymorphisms (SNPs) which may alter p53 protein function [6]. Several functional p53 SNPs have been reported to be associated with risk of developing different human cancers, including lung cancer [7-9].

P53 rs2078486 SNP was recently identified to be associated with lung cancer risk in lifetime never smokers in a pathway-based genotyping study which evaluated a comprehensive panel of 11,737 SNPs in inflammatory-pathway genes [10]. One case-control study conducted in Los Angeles found elevated lung cancer risk associated with the variant genotype of p53 rs2078486 SNP (unpublished doctoral dissertation from Yi Ren Wang). However this association was not
confirmed by another pooled genome-wide association study [11]. To our knowledge, no case-control study has been conducted in the Asian population to replicate this association.

The most studied p53 SNP rs1042522 is characterized by substitution of Arginine (Arg) by Proline (Pro) at codon 72 (G12139C, Arg72Pro) and may noticeably affect p53 function [12]. However, very few studies examined if there are interactions between Arg72Pro polymorphism and smoking or other lifestyle factors on lung cancer risk.

A case-control study was conducted to examine the associations of p53 rs2078486 and rs1042522 SNPs with lung cancer risk in a Chinese population and further explore their interactions with some demographic and lifestyle factors.

Methods

Study participants

A case-control study was conducted between 2005 and 2007 in Taiyuan city, the capital of Shanxi province, China. Prior to the initiation of the recruitment, IRB approvals were obtained from Fudan University (IRB#04-10-0022) and UCLA (IRB#11-003153), respectively. Lung cancer cases were enrolled from the Shanxi tumor hospital, which admitted about 70% of the cancer patients from the city. Eligible cases were newly diagnosed lung cancer cases, 20 years of age or older, lived in Taiyuan city for 10 years or more, in stable medical condition and willing to participate. Controls were randomly selected from 13 communities in Taiyuan city. Eligible controls were 20 years of age or older, must have lived in Taiyuan city for 10 years or more, and had no history of cancer or any other serious chronic diseases. A total of 399 lung cancer patients and 466 healthy controls were recruited to participate in this study. Response rates were 89% for
eligible cases and 85% for eligible controls. Written informed consent was obtained from all study participants.

Data collection

All cases and controls were interviewed by professional staff to collect information on demographic factors, dietary and cooking habits, active and passive smoking history, alcohol drinking habits, tea drinking habits, residence and housing history, occupational history and related exposure, physical activities and disease history.

Blood sample collection and laboratory analysis of gene polymorphisms

Blood samples were collected from 97.9% of cases and 98.9% of controls. Serum and blood clot were immediately separated and all samples were stored in freezer at -80 °C. Genomic DNA was extracted using a modified phenol-chloroform protocol. Genotyping was performed in the Molecular Epidemiology Laboratory at Department of Epidemiology, School of Public Health at UCLA. P53 SNP genotyping was performed using Sequenom platform (Sequenom, Inc., San Diego, CA). Polymerase chain reaction (PCR) and extension primers were designed using MassARRAY Assay Design 3.1 software (Sequenom, Inc., San Diego, CA). Genotyping procedures were performed according to the manufacturer’s iPLEX Application Guide (Sequenom Inc. SanDiego,CA). For quality control, we included two negative controls (H₂O) in each 96-well plate. Around 4.5% of samples were selected for duplication and the concordance is 99.5%.

Definition of indoor air pollution index
An indoor air pollution index was created to integrate the impacts from different types of cooking and heating fuels, use of ventilator in kitchen, windows opening behaviors and secondhand smoke exposure at home on indoor air pollution levels. For each component of this index, a score of ‘0’ or ‘1’ represented low or high indoor air pollution, respectively. A summarized score lower than 2 was defined as low indoor air pollution exposure and higher or equal to 2 was defined as high indoor air pollution exposure [13].

**Statistical Analysis**

Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using unconditional logistic regression models to evaluate the independent effects of the two p53 SNPs. We present each of the associations in additive, dominant and recessive models, respectively. Potential confounding factors adjusted in the multivariate models included age, education level, annual personal income 10 years ago, pack-years of smoking, alcohol drinking and tea drinking status. Stratified analyses were conducted among subgroups with different age, gender, smoking status, alcohol and tea drinking status, indoor air pollution exposure and histo-pathological types of lung cancer. Multiplicative interactions of p53 SNPs with some lifestyle factors were assessed using ORs for interactions by including their product terms in the logistic regression models. Additive interactions were assessed using relative excess risk due to interaction (RERI), as described previously [14]. All statistical analyses were performed using SAS software (version 9.3). Associations were considered statistically significant if the p-value < 0.05 in the two-sided test.

**Results**
Basic characteristics of lung cancer cases and controls are presented in Table 1. No statistically significant differences in age and gender were found between cases and controls. Controls tended to have higher education levels, average annual income and body mass index than cases (p < 0.001). Lung cancer cases were more likely to be smokers and had higher pack-years of smoking, but were less likely to be current tea drinkers (p < 0.001) (Table 1).

Table 2 presents the independent associations between the two p53 SNPs and lung cancer risk in the total study population. No significant associations with lung cancer risk were found for p53 rs2078486 SNP, despite a tendency towards an elevated lung cancer risk associated with the variant genotype. A significantly increased lung cancer risk was observed among individuals with the homozygous variant genotype (CC) of p53 SNP rs1042522 (adjusted OR: 1.63, 95% CI: 1.10 - 2.41), compared with the homozygous wild type (GG). Adjusted ORs for rs1042522 were also statistically significant in the recessive model (adjusted OR: 1.57, 95% CI: 1.11- 2.21), but not in the dominant model. C allele of p53 SNP rs1042522 was significantly associated with increased risk of developing lung cancer (adjusted OR: 1.26, 95% CI: 1.04 - 1.53) (Table 2).

Results from the stratified analyses are presented in Figure 1. Presence of one or both copies of minor allele (TC or CC) of p53 rs2078486 SNP was significantly or borderline significantly associated with elevated lung cancer risk among older individuals (adjusted OR: 1.53, 95% CI: 0.97 - 2.41), smokers (adjusted OR: 1.70, 95% CI: 1.08 - 2.67), alcohol drinkers (adjusted OR: 2.41, 95% CI: 1.25 - 4.65) and individuals with high indoor air pollution exposure (adjusted OR: 1.51, 95% CI: 1.00-2.30) (Figure 1). Significant multiplicative and additive interactions were found between the indoor air pollution index and p53 rs2078486 polymorphism (adjusted OR for interaction: 1.89, 95% CI: 1.00-3.56, adjusted REPI: 0.90, 95% CI: 0.11-1.70). There was also some suggestive evidence of multiplicative interaction between smoking and p53 rs2078486
polymorphism (adjusted OR for interaction: 1.80, 95% CI: 0.99-3.30) and additive interaction (adjusted RERI: 2.49, 95% CI: -0.03, 5.01) (Table 3).

Elevated risk of lung cancer associated with homozygous variant genotype (CC) of p53 SNP rs1042522 were observed in each subgroup. No obvious difference was observed between smokers and nonsmokers (Figure 1). The variant genotype of p53 SNP rs1042522 tended to confer stronger deleterious effect for younger individuals, males, alcohol and tea drinkers, however neither multiplicative nor additive interactions were observed between p53 SNP rs1042522 and any lifestyle factors on lung cancer risk (Table 3).

**Discussion**

This case-control study confirmed elevated lung cancer risk associated with the variant allele (C) of p53 SNP rs1042522, and this study is among the first to report an increased lung cancer risk associated with variant genotype of p53 SNP rs2078486 in an Asian population. Moreover, we found synergetic effects of smoking and indoor air pollution exposure with p53 SNP rs2078486 on lung cancer risk.

Overwhelming evidence suggested that the p53 tumor suppressor gene is a central regulatory node of multiple cellular response pathways to endogenous or exogenous stresses [15]. P53 protein has demonstrated the capacity to regulate activity of key effectors of cellular processes, such as DNA repair, cell cycle arrest, senescence, and apoptosis [16, 17]. Functional inactivation of p53 pathways is thought to affect p53 signaling and further alter cancer risk [15, 18].

P53 rs2078486 SNP was suggested to be a novel p53 SNP. One pathway-based genotyping study conducted among nonsmokers found statistically significant association between p53 rs2078486
SNP and lung cancer [10]. In the present study, we found some suggestive evidence of elevated lung cancer risk associated with TC or CC genotypes of p53 rs2078486 SNP (adjusted OR: 1.22, 95% CI: 0.91 - 1.63) in the overall study participants. Our result was in similar direction with one population-based case-control study conducted in Los Angeles (adjusted OR: 1.61, 95% CI: 1.18 – 2.20) (unpublished doctoral dissertation from Yi Ren Wang). Another case-control study also suggested that variant genotype of p53 rs2078486 SNP was significantly associated with increased ovarian cancer risk [19]. Therefore, this suggests that there might be a functional difference among different genotypes of p53 rs2078486 SNP, which may affect the risk of developing various types of cancers.

Moreover, we found carrying the variant alleles of p53 rs2078486 SNP was significantly associated with elevated lung cancer risk in smokers (adjusted OR: 1.70, 95% CI: 1.08 - 2.67) and individuals with high indoor air pollution exposure (adjusted OR: 1.51, 95% CI: 1.00 - 2.30). Cigarette smoking and air pollution have been linked with high frequency of p53 mutations [20-23]. The positive interactions observed between p53 SNP rs2078486 with smoking and indoor air pollution exposure in our study might suggest that individuals carrying the variant genotype of p53 rs2078486 may have compromised p53 function and respond poorly to the adverse effects of smoking and air pollution, thus have an elevated risk of developing lung cancer. In addition, the elevated risk associated with high-risk genotypes p53 rs2078486 SNP was more evident for the small cell carcinoma, which has been more strongly linked to cigarette smoking than the other histo-pathological types of lung cancer.

Elevated lung cancer risk associated with the variant C allele of p53 SNP rs1042522 observed in this study was consistent with previous studies conducted among the Asian populations (summarized OR under recessive genetic model: 1.37, 95% CI: 1.20 – 1.57; homozygote...
comparison CC vs. GG: 1.34, 95% CI: 1.16 – 1.56) [8]. In the present study, we did not find heterogeneity of lung cancer risks associated with p53 SNP rs1042522 in smokers versus non-smokers, which was also consistent with a previous meta-analysis [8]. Very few prior studies have examined if demographic or other lifestyle factors might modify the association between p53 SNP rs1042522 and lung cancer. In this study, we did not find statistically significant interactions between lifestyle factors and p53 SNP rs1042522 on lung cancer risk.

One major limitation of the present study is that the relatively small sample size, especially in the stratified analyses, limited our ability to detect moderate interactions. Large-scale epidemiological studies are needed in the future to confirm our findings. In addition, the use of multiple stratified analyses increases the chance for spurious associations.

Conclusions

In conclusion, this case-control study provided preliminary evidence that p53 rs2078486 SNP is a novel p53 SNP that may affect lung cancer risk, especially among smokers and individuals with high indoor air pollution exposure. There is some further evidence of significant interactions between p53 rs2078486 SNP and smoking and indoor air pollution exposure on lung cancer risk. Further studies with larger sample size and in different study populations are warranted to confirm our findings.
List of abbreviations

Single-nucleotide polymorphisms (SNPs); odds ratios (ORs); 95% confidence intervals (95% CIs); relative excess risk due to interaction (RERI)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YL performed the statistical analysis and drafted the manuscript. SC carried out the genetic polymorphism tests and helped to draft the manuscript. RN, LL, BZ, JS and XH have made substantial contributions to fieldwork and data collection. CC helped to draft the manuscript. JL, JS and LC participated in the design and coordination of the study. SY and ZZ participated in the design and fieldwork of the study. LM oversaw the study design, results interpretation and manuscript drafting. All authors read and approved the final manuscript.

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References:


Figure 1
Additional files provided with this submission:

Additional file 1: P53 LuCa table March 25 2013.doc, 176K
http://www.biomedcentral.com/imedia/1519657392952909/supp1.doc