

RESEARCH ARTICLE

TP63 Gene Polymorphisms, Cooking Oil Fume Exposure and Risk of Lung Adenocarcinoma in Chinese Non-smoking Females

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Abstract

Background: Genetic polymorphisms of TP63 have been suggested to influence susceptibility to lung adenocarcinoma development in East Asian populations. This study aimed to investigate the relationship between common polymorphisms in the TP63 gene and the risk of lung adenocarcinoma, as well as interactions of the polymorphisms with environmental risk factors in Chinese non-smoking females. **Methods:** A case-control study of 260 cases and 318 controls was conducted. Data concerning demographic and risk factors were obtained for each subject. The genetic polymorphisms were determined by Taqman real-time PCR and statistical analyses were performed using SPSS software. **Results:** For 10937405, carriers of the CT genotype or at least one T allele (CT/TT) had lower risks of lung adenocarcinoma compared with the homozygous wild CC genotype in Chinese nonsmoking females (adjusted ORs were 0.68 and 0.69, 95% CIs were 0.48-0.97 and 0.50-0.97, *P* values were 0.033 and 0.030, respectively). Allele comparison showed that the T allele of rs10937405 was associated with a decreased risk of lung adenocarcinoma with an OR of 0.78 (95% CI=0.60-1.01, *P*=0.059). Our results showed that exposure to cooking oil fumes was associated with increased risk of lung adenocarcinoma in Chinese nonsmoking females (adjusted OR=1.58, 95% CI=1.11-2.25, *P*=0.011). However, we did not observe a significant interaction of cooking oil fumes and TP63 polymorphisms. **Conclusion:** TP63 polymorphism might be a genetic susceptibility factor for lung adenocarcinoma in Chinese non-smoking females, but no significant interaction was found with cooking oil fume exposure.

Keywords: TP63 gene - SNPs - cooking oil fumes - lung adenocarcinoma - female nonsmokers

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Introduction

Lung cancer remains a leading cause of cancer-related mortality in China, Asia and worldwide (Ferlay et al., 2010; Sheila et al., 2012; Chen et al., 2013). It is well known that smoking is the predominant risk factor of lung cancer. However, not all lung cancer patients have a history of smoking, it is estimated that 15% male and 53% female of lung cancer patients are nonsmokers (Couraud et al., 2012), which suggests that in addition to tobacco, other risk factors are likely involved in the etiology of lung cancer. Over past 20 years, it is estimated that the incidence of male and female lung cancer cases increased by 44% and 76% respectively (Parkin et al., 2005). Some studies show that females are more likely than males to have non-smoking-associated lung cancer (Wakelee et al., 2007). In this sense it is more important to investigate non-tobacco related risk factors of lung cancer in non-smoking female population. Adenocarcinoma accounts for about 40% of lung cancer patients, with a higher incidence in women. Adenocarcinoma is the most frequent type of lung cancer

occurring in those who have never smoked. The etiology of lung cancer for nonsmoking females is not very clear (Xiao et al., 2011).

As we know, many lung cancer patients do not have the history of smoking and a lot of smokers didn't develop lung cancer, suggesting that host susceptibility factors may play an important role in the development of lung cancer. TP63, a nuclear marker of myoepithelial cells, through a variety of mechanisms involved in oncogenesis. There is growing evidence that genetic variation in TP63 may influence susceptibility to lung adenocarcinoma in East Asian populations (Hosgood et al., 2012). Recent genome-wide association study (GWAS) datasets have shown that the single nucleotide polymorphisms (SNPs) of rs10937405 and rs4488809 in TP63 gene on locus 3q28 might be associated with lung adenocarcinoma susceptibility (Miki et al., 2010; Hu et al., 2011). However, above studies were among the whole population, without the results in different characteristic subjects and these studies did not involve the gene-environment interaction, which is considered as the key to understand the etiology

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of cancer. So we chose the non-smoking female population as study subjects and analyze the gene-environment interaction in the present study.

The effect of the interaction between polymorphisms in TP63 gene with environmental exposures on lung adenocarcinoma susceptibility in non-smoking females has not been reported so far. In order to provide further insights into the association of lung cancer with rs10937405 and rs4488809, we conducted this case-control study of lung adenocarcinoma in non-smoking female population in Shenyang City, P.R. China. We also investigated potential environmental risk factors and the interaction of susceptibility genes with environmental risk factors, which was valuable because positive findings would help understand cancer etiology and propose environmental modifications for disease prevention.

Materials and Methods

Study subjects and data collection

In this hospital-based case-control study, the case group consisted of 260 diagnosed nonsmoking female patients (between January 2004 and November 2009) with lung adenocarcinoma. At the same time, 318 controls were selected from cancer-free patients. Controls were all non-smoking females and frequency matched to cases on age (± 5 years). All subjects were unrelated ethnic Han Chinese. The study was approved by the Institutional Review Board of China Medical University. Informed consent was obtained from each participant or each participant's representatives if direct consent could not be obtained.

Individual with a total of 100 cigarettes in his lifetime was defined as a smoker, otherwise he was considered as a non-smoker. Every subject in the study donated 10ml blood for SNP detection and was investigated about demographic data and environmental exposure. Data concerning demographic characteristics, cooking oil fume exposure, fuel smoke exposure, passive smoking, family history of cancer, and et al. were obtained for each case and control.

DNA isolation and genotyping

Genomic DNA samples were isolated by Phenol-chloroform Method. Genotyping of the SNPs was done using Taqman® allelic discrimination (Applied Biosystems, Foster City, CA) with a commercially available primer probe set (assay ID C__32076701_10 for rs10937405 and C_248358_10 for rs4488809). When genotyping was performed, appropriate negative controls were included in each run. Quality control was done by randomly selecting 10% of subjects who were tested twice by different persons, and the results were found to be concordant for all of the masked duplicate sets.

Statistical analysis

Pearson's chi-square test was used to examine differences in environmental risk factors and SNP genotypes and alleles between cases and controls. Hardy-Weinberg equilibrium (HWE) of the genotypes was tested by performing a goodness-of-fit χ^2 test. Unconditional

Table 1. Genetic Polymorphisms and Lung Adenocarcinoma Risk

SNP	Cases n (%)	Controls n (%)	OR [95%CI] ^a	P value
rs10937405				
CC	157 (60.4)	164 (51.6)	1.00	-
CT	82 (31.5)	125 (39.3)	0.68 [0.48-0.97] ^b	0.033
TT	21 (8.1)	29 (9.1)	0.75 [0.41-1.36] ^b	0.341
CT/TT	103(39.6)	154(48.4)	0.69 [0.50-0.97] ^b	0.030
C	396 (76.2)	453 (71.2)	1.00	-
T	124 (23.8)	183(28.8)	0.78 [0.60-1.01] ^c	0.059
rs4488809				
TT	72 (27.7)	84 (26.4)	1.00	-
CT	121 (46.5)	156 (49.1)	0.90 [0.61-1.34] ^d	0.616
CC	67 (25.8)	78 (24.5)	0.99 [0.63-1.57] ^d	0.979
CT/CC	188 (72.3)	234 (73.6)	0.93 [0.65-1.35] ^d	0.716
T	265 (51.0)	324 (50.9)	1.00	-
C	255 (49.0)	312(49.1)	0.99 [0.79-1.26] ^e	0.995

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; ^aORs were calculated by unconditional logistic regression and adjusted for age; ^bOR and P value were calculated compared with CC genotype of rs10937405 polymorphism; ^cOR and P value were calculated compared with C allele of rs10937405 polymorphism; ^dOR and P value were calculated compared with wild genotype(TT) of rs4488809 polymorphism; ^eOR and P value were calculated compared with T allele of rs4488809 polymorphism

logistic regression analysis was performed to calculate the odds ratios (OR) and their 95% confidence intervals (CI) for evaluating the associations between SNP genotypes and environmental exposure with lung adenocarcinoma risks. The gene-environment interaction was assessed by crossover analysis and logistic regression models. All statistical analyses were performed using SPSS (v 12.0). All of the tests were two-sided and statistical significance was defined as $P < 0.05$.

Results

The present study comprised 260 lung adenocarcinoma cases and 318 controls, who were all nonsmoking females. The mean ages were 56.4 ± 11.6 and 57.0 ± 11.1 in case and control group, respectively. There were no difference in age between cases and controls ($t=0.653$, $P=0.514$). The minor allele frequencies of the rs10937405 T allele and rs4488809 C allele in the controls were 0.288 and 0.491, respectively. All allele distributions were consistent with Hardy-Weinberg equilibrium ($\chi^2=0.53$ and $P=0.465$ for rs10937405, $\chi^2=0.11$ and $P=0.741$ for rs4488809).

Genotype distribution and its association with lung adenocarcinoma susceptibility were shown in Table 1. For 10937405, heterozygous carriers of the CT genotype had a lower risk of lung adenocarcinoma compared with the homozygous wild genotype (adjusted OR=0.68, 95%CI=0.48-0.97, $P=0.033$). With the CC genotype of rs10937405 as reference group, after adjustment for age, carrying at least one T allele (CT/TT) was less likely to develop lung adenocarcinoma (adjusted OR=0.69, 95%CI=0.50-0.97, $P=0.030$). Further analyses were carried on by allele comparison and the T allele of rs10937405 was found to be associated with a decreased risk of lung adenocarcinoma with marginally significant OR of 0.78 (95%CI=0.60-1.01,

Table 2. Environmental Exposure and Lung Adenocarcinoma Risks

Variable	Cases n (%)	Controls n (%)	OR [95%CI] ^a	P value
Family history of cancer	36 (13.8)	33(10.5)	1.40[0.85-2.32]	0.192
Passive smoking	154 (59.2)	170 (53.5)	1.28[0.92-1.79]	0.145
Fuel smoke exposure	70 (26.9)	80 (25.2)	1.10[0.76-1.60]	0.609
Cooking oil fume exposure	95 (36.5)	85 (26.7)	1.58[1.11-2.25]	0.011

OR, odds ratio; CI, confidence interval; aORs were calculated by unconditional logistic regression and adjusted for age

Table 3. rs10937405 in Relation to Risk of Lung Adenocarcinoma, Stratified by Cooking Oil Fume Exposure

Group	Cases n (%)	Controls n (%)	OR [95%CI] [*]	P value
Cooking oil fume exposure				
Exposure				
CC	56 (58.9)	36 (42.4)	1.00	-
CT	31 (32.6)	39 (45.9)	0.52 [0.27-0.97]	0.040
TT	8 (8.4)	10 (11.8)	0.53 [0.19-1.48]	0.225
CT/TT	39(41.0)	49(57.7)	0.52 [0.29-0.94]	0.031
Non-exposure				
CC	101 (61.2)	128 (54.9)	1.00	-
CT	51 (30.9)	86 (36.9)	0.74 [0.48-1.14]	0.169
TT	13 (7.9)	19 (8.2)	0.84 [0.40-1.80]	0.659
CT/TT	64(38.8)	105(45.1)	0.76 [0.50-1.14]	0.179

OR, odds ratio; CI, confidence interval; *ORs and 95%CIs were calculated by logistic regression, with rs2736100 wild genotype (TT) as the reference group; ORs were adjusted for age

$P=0.059$). However, as for rs4488809, no association was found between its genotypes or alleles with the risk of lung adenocarcinoma.

Table 2 showed the distribution of environmental risk factors in the lung adenocarcinoma group and control group, as well as the associations between environmental exposures and lung adenocarcinoma risks. There were no significant associations between family history of cancer, passive smoking and fuel smoke exposure with lung adenocarcinoma risk. However, the cases were more likely than the controls to report cooking oil fume exposure (adjusted OR=1.58, 95%CI=1.11-2.25, $P=0.011$).

In the stratified analyses, we found that the decreased risk associated with rs10937405 variant genotypes (CT/TT) was more pronounced in individuals with exposure to cooking oil fume (adjusted OR=0.52, 95%CI=0.29-0.94, $P=0.031$) (Table 3). Among subjects exposure to cooking oil fume, the risk of lung adenocarcinoma was significantly lower in people with CT genotype (adjusted OR =0.52, 95%CI=0.27-0.97, $P=0.040$).

Finally, we analyzed the interaction of rs10937405 and cooking oil fume exposure. The hypothesis testing of crossover analysis and logistic model suggested that the gene-environment interaction was not statistically significant. From the results of our analysis, there may be no interaction between rs10937405 and cooking oil fume exposure in lung adenocarcinoma among nonsmoking females in China.

Discussion

Lung cancer is a chronic disease with multiple etiologies, which are not clear now. As we know, cigarette smoking cannot fully explain the etiology of lung cancer

in Chinese women, who smoke rarely but develop lung cancer more often. The non-smoking females are the desired population to explore the unknown important environmental factors of lung cancer, besides tobacco smoking, as well as the gene-environment interaction. So we chose the non-smoking female population as the study subjects to analyze the association of lung adenocarcinoma risk with genetic polymorphisms in TP63 gene, as well as the environmental risk factors and gene-environment interaction.

In the present study, we included genetic polymorphisms of TP63 (rs10937405 and rs4488809) and some environmental risk factors. We have replicated the association between lung adenocarcinoma and SNP rs10937405. The association of this SNP with lung cancer risk was previously reported by a GWAS conducted in Japan and South Korea (Miki et al., 2010), and then be studied in the women who have never smoked tobacco (Hosgood et al., 2012). To be accordant with Hosgood III (Hosgood et al., 2012), TP63 rs10937405 showed a stronger association with lung adenocarcinoma in our population than did TP63 rs4488809. A recent GWAS study among Han Chinese population also found that genetic variation in the TP63 region was associated with lung cancer risk, which showed more pronounced association for rs4488809, although the results were not reported for nonsmoking females (Hu et al., 2011). In previous GWAS conducted among subjects of European descent, lung cancer risk was not associated with TP63 variants (Amos et al., 2008; Hung et al., 2008; McKay et al., 2008; Wang et al., 2008; Landi et al., 2009). A GWAS carried out in Europe reported a suggestive but weak association between lung cancer and TP63 rs4488809 (Hung et al., 2008). The reason for diverse association found in Caucasians and East Asians may be the different ethnic background and environmental exposures in North America and Europe compared to those in East Asia.

P63, the product of the TP63 gene, is an important member of p53 family. TP63 is expressed mainly including two isoforms of the TA form which is tumor suppressor and N-terminal-truncated (Δ N) form which is oncogene (Hibi et al., 2000). Expression of the TAp63 isoforms are transcribed by a promoter located upstream of exon 1, whereas Δ Np63 isoforms are regulated by a promoter located in intron 3 (Moll et al., 2004). Because possible candidate SNPs are located in intron 1 of TP63, which encodes TAp63 isoforms, we conclude that one or more SNPs in this region may have a functional role in the regulation of TP63 gene expression. We found rs10937405 in this region was significantly associated with lung adenocarcinoma, suggesting its possible functional role. In contrast to the results of other studies in Japan, Korea and China (Miki et al., 2010; Hu et al., 2011), there was no association between rs4488809 and lung adenocarcinoma in our study. In view of such different results, the relationship between rs4488809 and lung adenocarcinoma should be confirmed by larger sample size population study and functional analyses.

All of above studies did not analyze the gene-environment interaction in the development of lung cancer, which is considered more important in the etiology of lung cancer. In the present study, we have analyzed the possible environmental exposures associated with

lung adenocarcinoma in Chinese nonsmoking females, as well as the interaction of TP63 SNPs and environmental exposures. Our results showed that nonsmoking female lung adenocarcinoma was closely related to cooking oil fumes exposure. There are studies in Shanghai, Taiwan, Hong Kong and other places reporting the significant association of lung cancer in women with exposure to cooking oil fume (Zhong et al., 1999; Ko et al., 2000; Yu et al., 2006; Pan et al., 2008). Previous studies have shown that sugar, proteins, fat and amino acids can release some harmful substances, such as particulate matter (PM), polycyclic aromatic hydrocarbons, aromatic amines, nitro-PAHs, and aldehydes under the high-temperature treatment (Vainiotalo et al., 1993; Li et al., 1994; Wu et al., 1998; Chiang et al., 1999; Lund et al., 2006). Laboratory studies have demonstrated that cooking oil fumes condensates can induce DNA damages and oxidative stress in lung epithelial cells (Tung et al., 2001; Wu et al., 2002). This might explain why the cooking oil fumes exposure could increase lung cancer risk.

We have firstly reported that there was not significant interaction of rs10937405 and cooking oil fumes exposure in the development of lung adenocarcinoma among Chinese nonsmoking female population in this study. After stratified analysis, we found that the decreased risk associated with rs10937405 variant genotypes was more pronounced in individuals with exposure to cooking oil fume. The cross-over analysis and logistic results suggested that no statistically significant interaction was found between TP63 gene polymorphism and cooking oil fume exposure. Because it is just a statistical estimation, larger sample size studies and further studies concerning their biological validity are required.

In summary, this study reported the association of rs10937405 polymorphism in TP63 gene with the risk of lung adenocarcinoma in Chinese nonsmoking female population, and firstly studied the interaction of TP63 polymorphism with the environmental risk factor in the development of lung adenocarcinoma, suggesting that rs10937405 constitutes an important factor for nonsmoking female with a genetic predisposition to lung adenocarcinoma. Future well-designed and larger population studies are of great value to confirm these findings.

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