

RESEARCH ARTICLE

CHRNA5 rs16969968 Polymorphism Association with Risk of Lung Cancer - Evidence from 17,962 Lung Cancer Cases and 77,216 Control Subjects

Zhi-Wei Xu^{1&}, Guan-Nan Wang^{2&}, Zhou-Zhou Dong¹, Tao-Hong Li¹, Chao Cao^{3*}, Yu-Hong Jin^{1*}

Abstract

Background: Genetic studies have shown a possible relationship between the rs16969968 polymorphism in CHRNA5 and the risk of lung cancer. However, the results have been conflicting. Thus we rigorously conducted a meta-analysis to clarify any association. **Materials and Methods:** A total of 10 case-control studies involving 17,962 lung cancer cases and 77,216 control subjects were analysed. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to measure the strength of the association. **Results:** We found the CHRNA5 rs16969968 polymorphism to be associated with the risk of lung cancer (AA vs GG: OR=1.60, 95% CI=1.51-1.71). On stratified analysis by smoking status, a statistically significant increased risk was observed in the smoking group (AA vs GG: OR=1.80, 95% CI=1.61-2.01). However, this polymorphism was not associated with lung cancer risk in Asians (AA vs GG: OR=0.95, 95% CI=0.35-2.59), whereas it was linked to increased risk of lung cancer among Caucasians (AA vs GG: OR=1.65, 95% CI=1.55-1.76). **Conclusions:** Our meta-analysis provided statistical evidence for a strong association between rs16969968 polymorphism and the risk of lung cancer, especially in smokers and Caucasians. Application of this relationship may contribute to identification of individuals at high risk of lung cancer and indicate a chemoprevention target.

Keywords: rs16969968 - polymorphism - lung cancer - CHRNA5 - meta-analysis

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Introduction

Lung cancer is one of the leading causes of malignancy-related death and has become a major public health problem worldwide. It accounts for 17% of all new cancer cases and kills more people than any other cancer (Jemal et al., 2011). The exact underlying molecular mechanisms of lung cancer remain unknown. Although tobacco smoking and exposure to several occupational and environmental carcinogens are known risk factors for lung cancer, they are insufficient to explain the different lung cancer morbidity in the same exposure. Therefore, some host factors including genetic polymorphism have attracted significant interest in the study of pulmonary tumorigenesis (Toh et al., 2006; Ahmad et al., 2015).

The subunits CHRNA5/A3/B4 on chromosome 15q25 are considered to be correlated with smoking-related disease and nicotine addiction (Weiss et al., 2008; Saccone et al., 2009; Ware et al., 2011). These subunits are well known to encode the nicotine-acetylcholine receptors (nAChRs), which are the original physiological product in the central and peripheral nervous systems after

smoking tobacco. Moreover, nicotine can induce cellular proliferation, tumour invasion, and angiogenesis, and it inhibits apoptosis mediated by nAChRs (Dasgupta et al., 2006; Dasgupta et al., 2009; Liu et al., 2010). In addition, Falvella et al. (2009) detected an obvious up-regulation of the CHRNA5 gene in lung tumour tissue. Thus, single nucleotide polymorphism (SNP) variant in CHRNA5 may influence the strength of cancer risk.

SNP at rs16969968 leads to the transposition of aspartic acid (G allele) to asparagine (A allele) at amino-acid position 398 (D398N) of the CHRNA5 protein (Weiss et al., 2008). Recently, the relationship between genetic variants of the rs16969968 in CHRNA5 and lung cancer risk has drawn tremendous attention. A study carried out on a Norwegian population implicated a more than 2-fold increased risk of lung cancer associated with the AA genotype carriers compared with the carriers of the GG genotype (Gabrielsen et al., 2013). However, in another report, the investigators demonstrated that CHRNA5 rs16969968 might have a limit effect on the susceptibility to lung cancer (Islam et al., 2013). Given these conflicting results, it is essential to perform a quantitative synthesis of

¹Department of Critical Care Medicine, ²Department of Gynaecology, Ningbo Medical Center, Lihuili Hospital, ³Department of Respiratory Medicine, Affiliated Hospital of School of Medicine, Ningbo University, Ningbo, China &Equal contributors *For correspondence: caoctor@163.com, yuhongjin@sina.com

the evidence with rigorous methods. We conducted a meta-analysis on published large-scale case-control studies to evaluate the association between the rs16969968 polymorphism and the susceptibility of lung cancer.

Materials and Methods

Publication search strategy

We searched MEDLINE, Web of Science, and EMBASE databases to identify studies published before June 2015 using combinations of the search terms: (rs16969968 OR D398N) AND (gene OR polymorphism OR genetic variant) AND (lung cancer). Trials were not excluded on the basis of language. All available studies were retrieved. The abstracts of relevant scientific meetings were also examined to ensure complete review of the available studies. If genotype frequency data were not provided in the published articles, we attempted to contact the corresponding author for additional studies and the missing data.

Selection criteria

The specific inclusion criteria to the meta-analysis were as follows: case-control studies, estimated correlations of the rs16969968 polymorphism and lung cancer risk, supply of the available genotype frequency both in case and control groups, and adequate published data for evaluating odds ratios (ORs) with 95% confidence intervals (CIs). Only large-scale case-control studies with a minimum of 100 subjects were included in our meta-analysis.

Data extraction

Two authors independently extracted data and reached a consensus on all of the available items, including the first author (ref.), published year, ethnicity (each of the ethnic groups categorized as Caucasians, Asians, etc.), definition of cases, genotype determination methods, smoking conditions, matching situations, and genotyping information. Firstly, we considered the allele comparison model (A vs. G), homozygous genotype comparison (AA vs. GG), heterozygote genotype comparison (AG vs. GG), recessive effect model comparison (AA vs. AG + GG), and dominant effect model comparison (AA + AG vs. GG). In addition, subgroup analyses were conducted according to smoking status and ethnicity (Caucasians vs. Asians).

Statistical analysis

This meta-analysis was used the Hardy–Weinberg equilibrium (HWE) to measure the frequencies of the genotype about every control group compared with expected genotype. OR and 95%CI of each case-control group were used to estimate the intension of correlation of rs16969968 polymorphisms with lung cancer risk. The Q statistic test was performed to evaluate the heterogeneity between individual studies, and $p < 0.1$ was considered significant. The I^2 test was used to measure the strength of the heterogeneity, with $I^2 = 0$ representing absolute consistency and $I^2 < 25%$, 25 - 75%, and $> 75%$ representing low, moderate, and high degrees of inconsistency, respectively (Higgins et al., 2003; Cao et al., 2012). The fixed-effect model was selected when the strength was assumed to be homogeneous; otherwise, the random-effect model was used. We also performed the Z-test to determine the significance of the combined OR; $P < 0.05$ was regarded as significant. Egger's test and a funnel plot were used to assess publication bias (Egger et al., 1997; Higgins and Thompson, 2002). All data were analysed with Stata (Version 12.0, Stata Corporation) and Review Manager (Version 5.0.24, the Cochrane Collaboration), and all of the P values were two-sided.

Results

Characteristics of included studies

The detailed steps for selecting studies process are shown in Figure 1. A total of 50 studies concerning the rs16969968 polymorphism in CHRNA5 with lung cancer were initially searched and screened for full text. Twenty-one studies were considered as potentiality. Of these, eleven studies were excluded due to data was

Table 1. Meta-analysis of rs16969968 Polymorphism on Lung Cancer Risk

Variables	No.a	Cases /Controls	A vs G			AA vs GG			AA vs AG+GG			AG vs GG			AA+AG vs GG		
			OR (95%CI)	Pb	P	OR (95%CI)	Pb	P	OR (95%CI)	Pb	P	OR (95%CI)	Pb	P	OR (95%CI)	Pb	P
Total	10	17962/77216	1.28 [1.24, 1.31]	0.59	0	1.60 [1.51, 1.71]	0.32	0	1.42 [1.34, 1.51]	0.65	0	1.27 [1.22, 1.32]	0.54	0	1.33 [1.28, 1.39]	0.44	0
Caucasian	9	16165/74984	1.29 [1.25, 1.33]	0.69	0	1.65 [1.55, 1.76]	0.55	0	1.42 [1.34, 1.51]	0.57	0	1.31 [1.25, 1.37]	0.69	0	1.37 [1.32, 1.43]	0.75	0
Asian	2	1797/2232	1.02 [0.81, 1.28]	0.25	0.86	0.95 [0.35, 2.59]	0.29	0.92	0.90 [0.33, 2.43]	0.34	0.84	1.04 [0.81, 1.33]	0.32	0.75	1.03 [0.81, 1.31]	0.26	0.82
Smoking status																	
Smoker	6	5402/34731	1.32 [1.26, 1.39]	0.73	0	1.80 [1.61, 2.01]	0.62	0	1.55 [1.40, 1.72]	0.72	0	1.30 [1.20, 1.40]	0.77	0	1.39 [1.29, 1.49]	0.83	0
Non-smoker	1	76/27454	1.10 [0.79, 1.55]	NA	0.57	1.06 [0.48, 2.34]	NA	0.89	0.93 [0.46, 1.89]	NA	0.84	1.28 [0.79, 2.06]	NA	0.31	1.23 [0.78, 1.94]	NA	0.37

a number of studies included; b P value for heterogeneity test. Fixed-effects model was used when P value for heterogeneity test > 0.10 ; NA: not applicable

not available (Hung et al., 2008; Shiraishi et al., 2009; Carcereny et al., 2010; Hansen et al., 2010; Sasaki et al., 2010; Yang et al., 2010; Wojas-Krawczyk et al., 2012; Spitz et al., 2013; Walsh et al., 2013; He et al., 2014) and one study was duplicate publications (Timofeeva et al., 2011). Ten articles (Young et al., 2008; Falvella et al., 2009; Zienolddiny et al., 2009; Lips et al., 2010; Truong et al., 2010; Jaworowska et al., 2011; Sakoda et al., 2011; Wei et al., 2011; Gabrielsen et al., 2013; Islam et al., 2013) met the inclusion criteria. The basic characteristics and genotype prevalence of these articles are shown in Table 1. Lung cancer cases were mostly histologically or cytologically diagnosed, and controls were free of cancer.

Quantitative synthesis

Ten studies including 17,962 lung cancer patients and 77,216 control subjects were used to assess the association

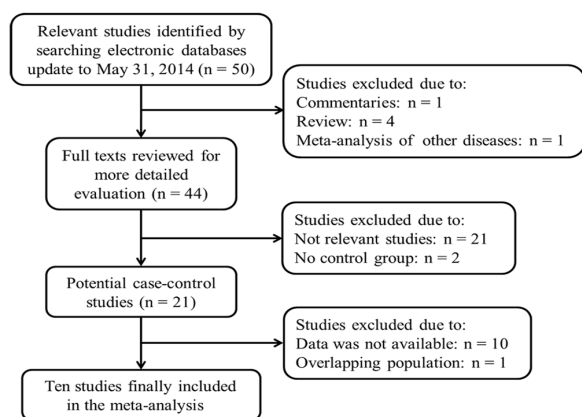


Figure 1. Literature Search Flow for rs16969968 Polymorphism and Risk of Lung Cancer

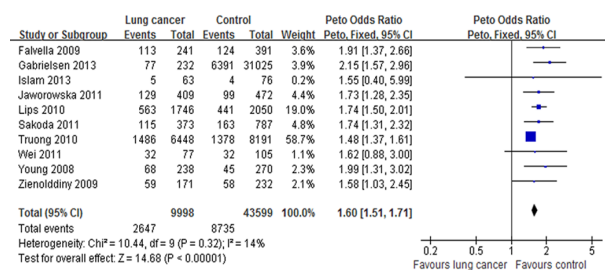


Figure 2. Meta-analysis of the Association between rs16969968 Polymorphism and Risk of Lung Cancer (AA vs GG)

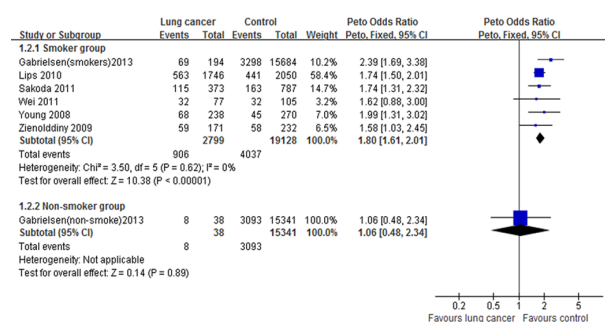


Figure 3. Meta-analysis of the Association between rs16969968 Polymorphism and Risk of Lung Cancer Stratified by Smoking Status (AA vs GG)

between rs16969968 polymorphism and lung cancer risk. A statistically significant association between lung cancer and rs16969968 polymorphism was found under homozygote comparison (OR=1.60, 95%CI=1.51-1.71, P<0.00001; P=0.32, I²=14% for heterogeneity) (Figure 2 and Table 2). Similar results were observed in the other gene models tested: allele comparison (OR=1.28, 95%CI=1.24-1.31, P<0.00001; P=0.59, I²=0% for heterogeneity), dominant genetic model (OR=1.33, 95%CI=1.28-1.39, P<0.00001; P=0.44, I²=0% for heterogeneity), recessive genetic model (OR=1.42, 95%CI=1.34-1.51, P<0.00001; P=0.65, I²=0% for heterogeneity), and heterozygote comparison (OR=1.27, 95%CI=1.22-1.32, P<0.00001; P=0.54, I²=0% for heterogeneity).

The effect of rs16969968 genotype on lung cancer risk was also evaluated in stratified analysis by smoking status. A statistically significant association between the rs16969968 genotype and lung cancer risk in the smoker group was found in all the gene models tested (homozygote comparison: OR=1.80, 95%CI=1.61-2.01, P<0.00001; P=0.62, I²=0% for heterogeneity). However, there was no association between rs16969968 and lung cancer risk in the non-smoker group (homozygote comparison: OR=1.06, 95%CI=0.48-2.34) (Figure 3).

In the stratification analyses for ethnicity, the effect of rs16969968 genotype on lung cancer risk increased with statistical significance in Caucasians under all genetic models (homozygote comparison: OR=1.65, 95%CI=1.55-1.76, P<0.00001; P=0.55, I²=0% for heterogeneity). However, this polymorphism was not associated with risk of lung cancer in Asians (homozygote comparison: OR=0.95, 95%CI=0.35-2.59) (Table 2 and Figure 4).

Sensitivity analysis

We performed sensitivity analysis sequentially by omission of individual studies. None of the pooled ORs were significantly influenced by any single study in the whole cohort or stratified analysis. The distribution of genotypes in the controls of Wei et al (Wei et al., 2011) was not consistent with Hardy-Weinberg equilibrium. When this study was excluded, the pooled OR was not significant affected (homozygote comparison: OR=1.60, 95%CI=1.50-1.71, P<0.00001; P=0.24, I²=23% for heterogeneity).

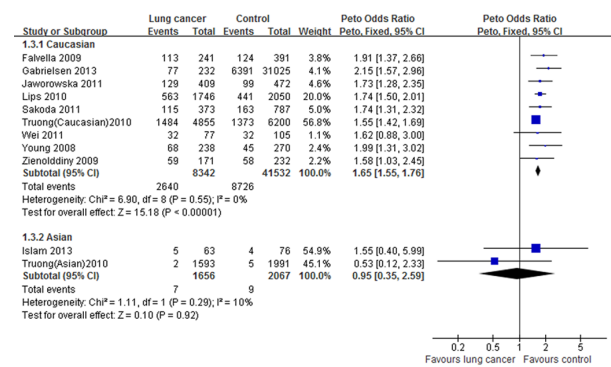


Figure 4. Meta-analysis of the Association between rs16969968 Polymorphism and Risk of Lung Cancer Stratified by Ethnicity (AA vs GG)

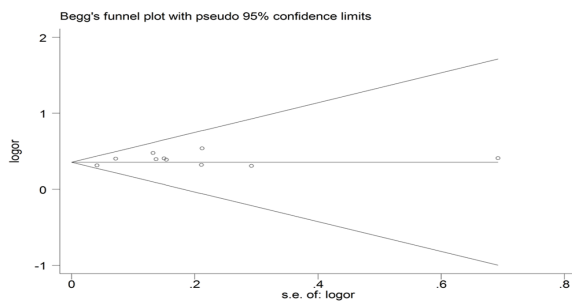


Figure 5. Funnel Plot of rs16969968 A/G Polymorphism and Risk of Lung Cancer (AA vs GG)

Publication Bias

Funnel plots and Egger's test were used to measure the publication bias of the studies. The data provided no evidence of obvious asymmetry in all genotypes in the overall population, and the results showed no publication bias (homozygote comparison: $t=1.96$, $P=0.086$) (Figure 5).

Discussion

Lung cancer is one of the malignant tumours with the highest incidence in the world. In recent years, we continued to focus on lung cancer and identified some biomarkers for lung cancer diagnosis (Cao et al., 2013) (Cao et al., 2013; Chen et al., 2014). Considering that biomarkers are regulated by the related gene, thus, investigating specific genetic differences in lung cancer might have more significant implications. Recently, rs16969968 polymorphism in CHRNA5 and the risk of lung cancer have drawn increasing attention, but the results have been inconsistent. We carried out a large-scale meta-analysis with a total of 17,962 lung cancer cases and 77,216 control subjects. The results from our study showed that patients with AA genotype of rs16969968 have a 1.60-fold higher risk for the development of lung cancer than that of GG genotype.

The findings of this study might have some alternative explanations. The potential gene-environment interactions should be considered. In a genome-wide association study, Sacconeri et al. initially demonstrated that rs16969968 in CHRNA5 is related to nicotine dependence (Saccone et al., 2007). Subsequently, those findings were supported by several studies (Bierut et al., 2008; Hung et al., 2008). It is generally accepted that tobacco smoking is a major risk factor for the development of lung cancer. Interestingly, the levels of plasma nicotine and tobacco-specific carcinogens among AA carriers are higher than those of non-carriers with the same smoking exposure (Le Marchand et al., 2008). It is possible that smokers can adjust the nicotine intake dose from a cigarette by altering their puffing time and depth. That is to say, smokers with the AA genotype inhale more toxicants by smoking the same cigarettes than non-carriers. Furthermore, Fowler et al. reported that this gene was involved in nicotine self-administration. It was reported that nicotine intake was obviously increased in mice with a null mutation in CHRNA5 with preclinical work (Fowler et al., 2011). In other words, individuals that carried the AA genotype of

rs16969968 increased their lung cancer risk by altering nicotine self-administration (MacQueen et al., 2014). On the other hand, another plausible explanation is that the variant of rs16969968 alters mRNA expression of CHRNA5. Falvella et al. (Falvella et al., 2009) revealed that CHRNA5 proteins express in tumour cells but not in stromal cells through immunohistochemical analysis. The relationship between this polymorphism and lung cancer remained significant after adjustment for cigarette smoking. Moreover, the researchers observed an increased risk of lung cancer in those who never smoked (Lips et al., 2010). This evidence supports that rs16969968 directly affects lung cancer risk.

As expected, the stratification analysis by smoking showed a remarkable association between the rs16969968 genotype and lung cancer. The lung cancer risk regarding genotype was found to be increased in the smoker group (OR=1.80, 95%CI=1.61-2.01), whereas little association was observed in non-smokers (OR=1.06, 95%CI=0.48-2.34). In the stratified analysis by ethnicity, AA genotype carriers were found to have 1.65-fold higher risk of suffering lung cancer compared with GG carriers in Caucasians (OR=1.65, 95%CI=1.55-1.76), but not for Asians (OR=0.95, 95%CI=0.35-2.59). The results may be due to the difference in ethnicity, and environmental exposures may affect allele frequency in rs16969968, which modified the risk of lung cancer. However, the findings in non-smokers and Asians should be interpreted with caution, because there were only one or two studies involving in the relevant populations. More studies based on larger populations should be conducted to further confirm these findings.

There were some limitations inherent in the study design. For instance, only a few studies were conducted to investigate the polymorphism and risk of lung cancer in non-smokers and in Asian populations. Therefore, there was insufficient power to support some conclusions in the subgroup analyses. Furthermore, our further valuation of potential gene-gene and gene-environment interactions was limited by the lack of original data.

There are several advantages in this meta-analysis. Firstly, only large-scale studies were included in our study, and some of them were genome-wide association study (GWAS) (Lips et al., 2010; Wei et al., 2011). The results of this meta-analysis were consistent with the findings of the genome-wide studies publications. Secondly, all studies were case-control research and contained the available genotype frequency. Thirdly, almost of all of these studies were histologically or cytologically diagnosed, and controls were free of cancer. Notably, the control subjects of most studies were well matched with the case patients regarding age, race, sex, and smoking status. Fourthly, there was no significant heterogeneity in all genetic contrasts.

To our best knowledge, this study is the first to synthetically analysis to investigate the association between CHRNA5 rs16969968 polymorphism and the risk of lung cancer. We demonstrated that the rs16969968 polymorphism was associated with lung cancer risk. Furthermore, similar results were observed in smokers and among Caucasians. Further large-scale research is

anticipated to verify our findings, and particularly, studies among different populations and non-smokers with lung cancer should be conducted in future research. Demonstrating this relationship may contribute to identifying individuals with high risk or indicate chemoprevention targets.

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