CYP2E1 Rsa I/Pst I polymorphism and lung cancer susceptibility: a meta-analysis involving 10,947 subjects

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Abstract

Many studies have examined the association between the CYP2E1 Rsa I/Pst I (rs3813867) polymorphism gene polymorphisms and lung cancer risk in various populations, but their results have been inconsistent. The PubMed and CNKI database was searched for case-control studies published up to October 2013. Data were extracted and pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated. In this meta-analysis, we assessed 23 published studies involving comprising 4727 lung cancer cases and 6220 controls of the association between CYP2E1 Rsa I/Pst I polymorphism and lung cancer risk. For the homozygote c2/c2 and c2 allele carriers (c1/c2 + c2/c2), the pooled ORs for all studies were 0.73 (95% CI = 0.62–0.84; P = 0.005 for heterogeneity) and 0.84 (95% CI = 0.77–0.92; P = 0.001 for heterogeneity) when compared with the homozygous wild-type genotype (c1/c1). In the stratified analysis by ethnicity, the same significantly risks were found among Asians and mixed population for both the c2 allele carriers and homozygote c2/c2. However, no significant associations were found in Caucasian population all genetic models. This updated meta-analysis suggests that CYP2E1 Rsa I/Pst I c2 allele is a decreased risk factor for the developing lung cancer among Asians and mixed population.

Keywords: CYP2E1 polymorphism lung cancer susceptibility meta-analysis

Introduction

Lung cancer remains the deadliest cancer worldwide despite improvements in diagnostic and therapeutic techniques [1]. Its incidence has yet to peak in many parts of world, particularly in China, which has become a major public health challenge [2]. The mechanism of lung carcinogenesis is still not fully understood. Besides smoking status established as the most important single factor in causing lung cancer, host factors, including genetic polymorphisms, have been some growing interest in the study of the tumorigenesis of lung cancer [3]. Many environmental carcinogens require metabolic activation by various drug-metabolizing enzymes.

In recent years, several drug-metabolizing enzymes have been identified as potential lung cancer susceptibility genes, such as cytochrome P450 1A1 (CYP 1A1) [4], Glutathione S-transferase [5, 6]. Cytochrome P450 2E1 (CYP2E1), a member of the cytochrome P450 superfamily, is a natural ethanol-inducible enzyme that is involved in the metabolic oxidation of low molecular weight carcinogens such as N-nitrosamines, ben-zene and vinyl chloride. CYP2E1 gene is located on 1q24.3-pter. It is 18,754 bp long consisting of nine exons and eight introns, which encodes a 493 amino acid protein [7, 8]. N-Nitrosamines are present in tobacco smoke, and activation of nitrosamines has been linked to the development of various cancers [9, 10]. The CYP2E1 (rs3813867) is reported to have genetic and racial variations that are caused by RsaI, PstI and DraI RELPs. The RsaI and PstI polymorphisms in the 5%-flanking (promoter) region of the gene are reported to affect the transcriptional activity of gene [11]. The predominant homozygous allele, the heterozygous allele and the homozygous rare allele of the RsaI/PstI (rs3813867) polymorphism are named the homozygous wild-type genotype (c1/c1), c2 allele carriers (c1/c2) and the rare homozygote (c2/c2), respectively.

Many studies have investigated associations between CYP2E1 (rs3813867) gene variation and lung cancer risk, but there is the perception that the findings have been inconsistent. A single study may be too underpowered to detect a possible small effect of the polymorphisms on lung cancer, especially when the sample size is relatively small. Besides, the past studies have not properly controlled for the potential confounding effect of smoking, the main risk determinant for lung cancer. Different types of study populations and study design may also contribute the disparate findings. Hence, we performed an updated meta-analysis of all eligible studies to derive a more precise estimation of the associations of Rsa I/Pst I polymorphisms with lung cancer.

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Materials and methods

Publication search

The electronic databases PubMed and CNKI (China National Knowledge Infrastructure) were searched for studies to include in the present meta-analysis, using the terms: ‘CYP2E1’, ‘P4502E1’, ‘polymorphism’ and ‘lung cancer’. An upper date limit of 1 October 2013 was applied; no lower date limit was used. The search was performed without any restrictions on language and was focused on studies that had been conducted in humans. We also reviewed the Cochrane Library for relevant articles. Concurrently, the reference lists of reviews and retrieved articles were searched manually. Only full-text articles were included. When the same patient population appeared in several publications, only the most recent or complete study was included in this meta-analysis.

Inclusion criteria

The included studies have to meet the following criteria: (i) evaluating the CYP2E1 Rsa I/Pst I polymorphism and lung cancer risk; (ii) case-control studies; and (iii) supply the number of individual genotypes for c1/c1, c1/c2, c2/c2 and/or c1/c2 + c2/c2 in lung cancer cases and controls, respectively.

Data extraction

Information was carefully extracted from all eligible publications independently by two authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors.

The following data were collected from each study: first author’s surname, year of publication, ethnicity, total numbers of cases and controls and numbers of cases and controls with the c1/c1, c1/c2, c2/c2 and/or c1/c2 + c2/c2 genotypes, respectively. If data from any of the above categories were not reported in the primary study, items were treated as ‘not applicable’. We did not contact the author of the primary study to request the information. Different ethnicity descents were categorized as Asian, Caucasian and mixed population. We did not require a minimum number of patients for a study to be included in our meta-analysis.

Statistical analysis

Odds ratio (OR) with 95% confidence intervals (CI) was used to assess the strength of association between the CYP2E1 Rsa I/Pst I polymorphism and lung cancer risk. The pooled ORs for the risk associated with the genotypes of homozygote c2/c2 and c2 allele carriers (c1/c2 + c2/c2) with the c1/c1 genotype were calculated. Subgroup analyses were done by ethnicity, pathological type and smoking status. Heterogeneity assumption was checked by the chi-square-based Q-test [12]. A P-value greater than 0.10 for the Q-test indicates a lack of heterogeneity among studies, so the pooled OR estimate of each study was calculated by the fixed-effects model (the Mantel–Haenszel method) [13]. Otherwise, the random-effects model (the DerSimonian and Laird method) was used [14]. One-way sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data-set to the pooled OR [15]. An estimate of potential publication bias was carried out by the funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was assessed by the method of Egger’s linear regression test, a linear regression approach to measure the funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the t-test suggested by Egger (P < 0.05 was considered representative of statistically significant publication bias) [16]. All calculations were performed with STATA version 11.0 (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

A total of 23 publications involving 4727 lung cancer cases and 6220 controls met the inclusion criteria and were ultimately analyzed [17–39]. Wu et al. [34] study conducted two sets of population (African-American, Mexican American), the two sets were classified as two independent studies in the meta-analysis through the discussion between the two authors. Table 1 presents the main characteristics of these studies. Among the 23 publications, 14 were published in English and 9 were in Chinese. The sample sizes ranged from 120 to 1052. Almost all of the cases were histologically confirmed. Controls were mainly healthy populations. There were 18 groups of Asians, 2 groups of Caucasians and 3 of mixed populations. Seven studies were hospital-based case–control studies and sixteen were population-based case–control studies.

Meta-analysis results

The main results of this meta-analysis are listed in Table 2. Overall, for the homozygote c2/c2 and c2 allele carriers (c1/c2 + c2/c2), the pooled ORs for all studies combined 4727 cases and 6220 controls were 0.73 (95% CI = 0.62–0.84, P = 0.005 for heterogeneity) and 0.64 (95% CI = 0.77–0.92; P = 0.001 for heterogeneity) (Fig. 1), when compared with the homozygous wild-type genotype (c1/c1). In the stratified analysis by ethnicity, significantly risks were found among Asians for both the c2 allele carriers (OR = 0.84, 95% CI = 0.77–0.92; P = 0.007 for heterogeneity) and homozygote c2/c2 (OR = 0.71; 95% CI = 0.61–0.82; P = 0.008 for heterogeneity). Similar results were found among mixed population in c2/c2 versus c1/c1 (OR = 0.75; 95% CI = 0.61–0.99; P = 0.418 for heterogeneity), and c2 allele carriers versus c1/c1 (OR = 0.70; 95% CI = 0.53–0.92; P = 0.594 for heterogeneity). However, among Caucasian populations, no significant association was found in c2/c2 versus c1/c1 (OR = 1.21; 95% CI = 0.84–1.92; P = 0.004 for heterogeneity), and c2 allele carriers versus c1/c1 (OR = 1.19; 95% CI = 0.83–1.71; P = 0.008 for heterogeneity).
In the subgroup analyses by pathological type, the ORs for the c2 allele carriers and the homozygote c2/c2 were 0.753 (95% CI = 0.68–0.87; $P = 0.427$ for heterogeneity) and 0.76 (95% CI = 0.60–0.97; $P = 0.006$ for heterogeneity) for lung SC. However, no significant associations were found in lung AC or SCLC for all genetic models. In the subgroup analyses by smoking status, there were no significant associations among smokers or non-smoker subgroup (Table 2).

**Table 1: Main characteristics of studies included in the meta-analysis**

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Ethnicity (country of origin)</th>
<th>Sample size (case-control)</th>
<th>Lung cancer case</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>c1/c1</td>
<td>c2/c2</td>
</tr>
<tr>
<td>Cao, 2013</td>
<td>Asian (Chinese)</td>
<td>526/526</td>
<td>386</td>
<td>NA</td>
</tr>
<tr>
<td>Li, 2012</td>
<td>Asian (Chinese)</td>
<td>217/198</td>
<td>116</td>
<td>NA</td>
</tr>
<tr>
<td>Eom, 2009 [21]</td>
<td>Asian (Korea)</td>
<td>387/387</td>
<td>254</td>
<td>NA</td>
</tr>
<tr>
<td>Zienolddiny, 2008</td>
<td>Caucasian (Norway)</td>
<td>311/343</td>
<td>248</td>
<td>14</td>
</tr>
<tr>
<td>Li, 2008 [23]</td>
<td>Asian (Chinese)</td>
<td>150/152</td>
<td>94</td>
<td>NA</td>
</tr>
<tr>
<td>Minegishi, 2007</td>
<td>Asian (Japanese)</td>
<td>505/256</td>
<td>300</td>
<td>30</td>
</tr>
<tr>
<td>Wang, 2006 [27]</td>
<td>Asian (Chinese)</td>
<td>91/91</td>
<td>61</td>
<td>7</td>
</tr>
<tr>
<td>Lee, 2006 [28]</td>
<td>Asian (Korea)</td>
<td>169/191</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>Liang, 2004 [29]</td>
<td>Asian (Chinese)</td>
<td>152/152</td>
<td>81</td>
<td>10</td>
</tr>
<tr>
<td>Shi, 2002 [31]</td>
<td>Asian (Chinese)</td>
<td>120/120</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td>Quinones, 2001</td>
<td>Mixed (Chilean)</td>
<td>59/148</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Li, 2000 [33]</td>
<td>Asian (Chinese)</td>
<td>92/137</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>Huang, 2000 [34]</td>
<td>Asian (Chinese)</td>
<td>54/260</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Wang, 1999 [35]</td>
<td>Asian (Chinese)</td>
<td>119/231</td>
<td>77</td>
<td>1</td>
</tr>
<tr>
<td>Qu, 1998 [37]</td>
<td>Asian (Chinese)</td>
<td>174/178</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>Wu, 1997 [38]</td>
<td>Mixed (African-American)</td>
<td>92/114</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>Wu, 1997 [38]</td>
<td>Mixed (Mexican American)</td>
<td>45/92</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>Watanabe, 1995</td>
<td>Asian (Japanese)</td>
<td>316/503</td>
<td>207</td>
<td>13</td>
</tr>
<tr>
<td>Persson, 1993 [41]</td>
<td>Caucasian (Swedish)</td>
<td>184/148</td>
<td>176</td>
<td>0</td>
</tr>
</tbody>
</table>

NA, not applicable.
eral cancers have drawn increasing attention. This meta-analysis genetic variants of the cytochrome P450 gene in the aetiology of several cancers has been a research focus in scientific community. Recently, accounted for this difference. Therefore, genetic susceptibility to cancers may have even with identical environmental exposure. Host factors, including there is a range of individual susceptibility to the same kind of cancer level of this polymorphic site can enhance the transcription and increase the has been shown to affect its transcription level. The variant type of polymorphisms, of which the RsaI/PstI polymorphism in its 5-flanking region CYP2E1 enzymatic activity in vitro. It is well recognized that there is a range of individual susceptibility to the same kind of cancer even with identical environmental exposure. Host factors, including polymorphisms of genes involved in carcinogenesis may have accounted for this difference. Therefore, genetic susceptibility to cancer has been a research focus in scientific community. Recently, genetic variants of the cytochrome P450 gene in the aetiology of several cancers have drawn increasing attention. This meta-analysis summarize all the available data on the association between CYP2E1 RsaI/PstI polymorphism and lung cancer risk, including a total of 4727 lung cancer cases and 6220 controls. Our results indicated a significant association between CYP2E1 RsaI/PstI polymorphism and lung cancer risk in Asians and mixed population. The c2 allele of CYP2E1 RsaI/PstI showed a decreased risk of lung cancer.

When stratified according to ethnicity, Asians and mixed population with the c2 allele carriers and the homozygote c2/c2 showed a decreased risk of lung cancer compared with those with the c1/c1 genotype. However, Caucasian population, no significant associations were found for all genetic models. These findings indicate that polymorphisms of CYP2E1 RsaI/PstI may be important in specific ethnicity of lung cancer patients and the effect of c2 allele on the risk of lung cancer may differ by ethnicity. Population stratification is an area of concern, and can lead to spurious evidence for the association between the marker and disease, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in [40]. In addition, it also likely that the observed ethnic differences may be due to the chance because studies with small sample size may have insufficient statistical power to detect a slight effect or may have generated a fluctuated assessment. Ethnicity is an important biological factor which may influence CYP2E1 functions through gene–gene interactions. The allelic frequencies were markedly different among ethnicities. The c2 allele of CYP2E1 RsaI/PstI polymorphism is more common in Asians than in the Western population [41]. Meanwhile, because the same polymorphism seemed to play different roles in lung cancer susceptibility among different ethnic populations and because the frequencies of single nucleotide polymorphisms were different among different ethnic groups, subgroup analyses based on ethnicity were conducted.

The mechanism for the rare allele RsaI/PstI polymorphism may reduce the lung cancer risk was not clear. CYP2E1 is involved in the metabolic activation of low molecular weight solvents and

### Table 2 Main results of pooled OR with CI in the meta-analysis

<table>
<thead>
<tr>
<th></th>
<th>Number of cases/controls</th>
<th>(c1/c2 + c2/c2) versus c1/c1</th>
<th>c2/c2 versus c1/c1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Total</td>
<td>4727/6220</td>
<td>0.84 (0.77–0.92)</td>
<td>0.001</td>
</tr>
<tr>
<td>Asian</td>
<td>3699/4921</td>
<td>0.84 (0.77–0.92)</td>
<td>0.001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>495/491</td>
<td>1.19 (0.83–1.71)</td>
<td>0.354</td>
</tr>
<tr>
<td>Mixed</td>
<td>533/808</td>
<td>0.70 (0.53–0.92)</td>
<td>0.011</td>
</tr>
<tr>
<td>SCC</td>
<td>496/882</td>
<td>0.753 (0.68–0.87)</td>
<td>0.023</td>
</tr>
<tr>
<td>AC</td>
<td>314/845</td>
<td>0.92 (0.77–1.43)</td>
<td>0.214</td>
</tr>
<tr>
<td>SCLC</td>
<td>215/468</td>
<td>1.18 (0.99–1.48)</td>
<td>0.125</td>
</tr>
<tr>
<td>Smoking</td>
<td>728/1523</td>
<td>0.89 (0.76–1.45)</td>
<td>0.125</td>
</tr>
<tr>
<td>No-smoking</td>
<td>489/1048</td>
<td>1.25 (0.89–1.58)</td>
<td>0.239</td>
</tr>
</tbody>
</table>

P (Q-test): P-value of Q-test for heterogeneity test; OR: odds ratio; CI: confidence interval.
procarcinogens including nitrosamines in tobacco, benzene, and halogenated hydrocarbons. *In vitro* expression studies indicate that the rare allele of *Rsa I/Pst I* polymorphism in the *CYP2E1* gene is associated with increased transcriptional activity [42]. However, the population studies on *CYP2E1* phenotypes have shown that individuals with the c2 allele of *Rsa I/Pst I* polymorphism have a lower basal *CYP2E1* activity and lower ethanol-induced enzyme activity [43]. Thus, the c2/c2 genotypes may have less ability to metabolically activate mutagens and carcinogens.

Some limitations of this meta-analysis should be acknowledged. Firstly, heterogeneity is a potential problem when interpreting all the results of meta-analyses. Although we minimized the likelihood by...
performing a careful search for published studies, using the explicit criteria for study inclusion, performing data extraction and data analysis strictly, the significant between-study heterogeneity still existed in almost each comparison. The presence of heterogeneity can result from differences in the selection of controls, age distribution, the lifestyle factors and so on. Although most of the controls were selected from healthy populations, some studies had selected controls among friends or family of lung cancer patients or patients with other diseases. Secondly, only published studies were included in this meta-analysis. The presence of publication bias indicates that non-significant or negative findings may be unpublished. Lastly, our results were based on unadjusted estimates, while a more precise analysis should be conducted if individual data were available, which would allow for the adjustment by other covariates including age, ethnicity, family history, environmental factors and lifestyle.

In conclusion, this meta-analysis suggests that the CYP2E1 Rsa I/Pst I polymorphism is associated with lung cancer risk, and the c2 allele is a decreased risk factor for developing lung cancer among Asians and mixed population. However, it is necessary to conduct large trials using standardized unbiased methods, homogeneous lung cancer patients and well matched controls, with the assessors blinded to the data.

Conflicts of interest
The authors confirm that there are no conflicts of interest.

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