Meta-Analysis of Low Density Lipoprotein Receptor (LDLR) rs2228671 Polymorphism and Coronary Heart Disease

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Received 29 January 2014; Revised 3 April 2014; Accepted 22 April 2014; Published 12 May 2014

Academic Editor: Hongwei Wang

1. Introduction

Coronary heart disease (CHD) is a complex disease caused by an insufficient blood flow inside the coronary vessels [1]. The blockage of the arteries is often caused by the plaque accumulated in the wall of arteries. The plaque is formed by excess low density lipoprotein cholesterol (LDL-C) in blood that dramatically increases the risk of CHD [2]. Low density lipoprotein receptor (LDLR) plays a key role in the regulation of cholesterol metabolism by removing excess LDL-C in blood [3, 4].

CHD is caused by both environmental and genetic factors [5]. Variations of genes involved in lipoprotein and lipid metabolism are playing an important role in the susceptibility of CHD [6]. LDLR gene mutations can lead to deficiency or abnormality of LDLR in the cell membrane surface and thus disrupt lipid metabolism [4]. LDLR gene mutations are known to cause familial hypercholesterolemia (FH) [2] that is an important risk factor of CHD and other atherosclerotic diseases [7]. Recent genome-wide association studies (GWASs) showed that LDLR gene mutations were significantly associated with the abnormal blood lipid levels and CHD [8, 9]. Among the LDLR polymorphisms, rs2228671 was associated with LDL-C levels and CHD in German and British populations [10–14]. However, discrepancies were also shown in the association of LDLR rs2228671 with CHD in Italian and Germans [15, 16].

Meta-analysis is able to combine and review the results from previous studies [17, 18]. Meta-analysis improves the power of comprehensive statistics by pooling the data from different studies. In the present study, we performed a meta-analysis of LDLR rs2228671 polymorphism with CHD among 17299 individuals in 8 studies.
2. Material and Methods

2.1. Retrieval of Studies and Selection Criteria. We systematically search available studies from 2003 to 2013 in PubMed (English), CNKI, and Wanfang (Chinese). Keywords were “coronary heart disease” or “coronary artery disease” or “myocardial infarction” combined with “LDLR” or “low density lipoprotein receptor” or “rs2228671” and “polymorphism” or “genetic association.” The inclusion criteria for the studies involved in this meta-analysis met the following criteria: (1) case-control study about LDLR rs2228671 polymorphism; (2) case-control study with genotyping or allelic information, or odd ratio (OR) with 95% confidential interval (CI).

2.2. Data Extraction. Data included in this meta-analysis was extracted independently from all studies using the same standard protocol by two reviewers (HY and YH). The inclusion criteria of our meta-analysis were as follows: first author’s name, publication year, ethnicity, numbers of cases and controls, genotype distribution, and OR with 95% CI.

2.3. Patients and Controls. The study protocol was approved by the ethical committee of School of Medicine, Ningbo University. A total of 162 cases and 113 controls were recruited in this study from the Affiliated Hospital of Ningbo University. All the participants in this study have signed the informed consent forms. All the 275 individuals underwent coronary angiography and were categorized into CHD patients and non-CHD controls according to our previous descriptions [5, 19]. All the participants enrolled in this study were Han Chinese residing in or near Ningbo city. None of individuals in this study had congenital heart disease, cardiomyopathy and severe liver, or kidney disease.

2.4. SNP Genotyping. Genomic DNA was isolated from peripheral blood lymphocytes using standard phenol-chloroform method and then was stored in TE buffer. All DNA samples were amplified by polymerase chain reaction (PCR). PCR was denatured at 94°C for 15 s, followed by 45 cycles of denaturation at 94°C for 20 s, annealing for 30 s at 56°C, extension at 72°C for 1 min, and a final extension at 72°C for 3 min. DNA amplification and genotyping was performed on the SEQUENOM Mass-ARRAY iPLEX platform according to the manufacturer’s instructions [5].

2.5. Statistical Analyses. Hardy-Weinberg equilibrium (HWE) was examined by the Arlequin program (version 3.5) [20]. The differences in the genotype and allele frequencies between cases and controls were analyzed by the CLUMP22 software with 10,000 Monte Carlo simulations [21]. Power analysis was performed by Power and Sample Size Calculation software [22]. Meta-analysis was made by REVMAN 5.0 (Cochrane Collaboration, Oxford, United Kingdom) and Strata 11.0 software (Strata Corporation, College Station, TX) [23, 24]. Publication bias was evaluated by Begg and Egger regression tests [25]. The combined ORs with 95% CI values were calculated by either fixed-effect or random-effect method [26]. A two-tailed P value of 0.05 or lower was defined to be statistically significant.

3. Results

We systematically searched in PubMed, CNKI, and Wanfang from 2003 to 2013, and selected a total of 57 literatures after removing the duplicated publications (Figure 1). According to the descriptions in the titles and abstracts, we excluded 26 irrelevant literatures, 6 literatures on other variants, and 12 literatures on other diseases. In addition, 1 literature without sufficient case-control genotyping data and 5 literatures...
Table 1: Characteristics of the association studies between rs2228671 and CHD.

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Ethnic group</th>
<th>Genotype (CC/CT/TT)</th>
<th>Cases</th>
<th>Controls</th>
<th>P-allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortlepp et al. (2003)</td>
<td>German</td>
<td>937/216/10</td>
<td>972</td>
<td>255/22</td>
<td>0.0453</td>
</tr>
<tr>
<td>Krawczak et al. (2006)</td>
<td>German</td>
<td>1755/379/19</td>
<td>1840</td>
<td>474/25</td>
<td>0.0184</td>
</tr>
<tr>
<td>Samani et al. (2007)</td>
<td>German</td>
<td>781/93/1</td>
<td>1417</td>
<td>224/3</td>
<td>0.0281</td>
</tr>
<tr>
<td>Samani et al. (2007)</td>
<td>British</td>
<td>1578/322/13</td>
<td>2332</td>
<td>569/34</td>
<td>0.0051</td>
</tr>
<tr>
<td>Schunkert et al. (2008)</td>
<td>German</td>
<td>236/43/2</td>
<td>224</td>
<td>61/5</td>
<td>0.0343</td>
</tr>
<tr>
<td>Erdmann et al. (2009)</td>
<td>German</td>
<td>282/64/3</td>
<td>671</td>
<td>164/15</td>
<td>0.3333</td>
</tr>
<tr>
<td>Martinelli et al. (2010)</td>
<td>Italian</td>
<td>549/134/9</td>
<td>227</td>
<td>61/3</td>
<td>0.73</td>
</tr>
<tr>
<td>Our study (2013)</td>
<td>Chinese</td>
<td>157/4/1</td>
<td>111</td>
<td>2/0</td>
<td>0.485</td>
</tr>
</tbody>
</table>

Table 2: Genotype and allele frequency distribution of LDLR gene rs2228671 polymorphism in cases and controls.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group</th>
<th>CC/CT/TT</th>
<th>χ²</th>
<th>P (df = 2)</th>
<th>C/T</th>
<th>χ²</th>
<th>P (df = 1)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Cases</td>
<td>157/4/1</td>
<td>0.86</td>
<td>1</td>
<td>318/6</td>
<td>0.87</td>
<td>0.49</td>
<td>0.47 (0.09–2.36)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>111/2/0</td>
<td></td>
<td></td>
<td>224/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Cases</td>
<td>113/2/1</td>
<td>0.51</td>
<td>0.77</td>
<td>228/4</td>
<td>NA</td>
<td>NA</td>
<td>0.49 (0.05–4.41)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>58/1/0</td>
<td></td>
<td></td>
<td>117/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Cases</td>
<td>44/2/0</td>
<td>0.53</td>
<td>0.76</td>
<td>90/2</td>
<td>NA</td>
<td>NA</td>
<td>0.42 (0.04–4.71)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>53/1/0</td>
<td></td>
<td></td>
<td>107/1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NA represents not analyzed; rs2228671 meets HWE in all groups (P > 0.05).

Table 3: Genotype and allele frequency distribution of LDLR gene rs2228671 polymorphism in European population.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group</th>
<th>CC/CT/TT</th>
<th>χ²</th>
<th>P (df = 2)</th>
<th>C/T</th>
<th>χ²</th>
<th>P (df = 1)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European population</td>
<td>Cases</td>
<td>6218/1251/57</td>
<td>20.59</td>
<td>&lt;.0001</td>
<td>13687/1365</td>
<td>20.26</td>
<td>&lt;.0001</td>
<td>1.180 (1.098–1.269)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>7685/1808/107</td>
<td></td>
<td></td>
<td>17178/2022</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

without detailed SNP information were also removed. At last, 6 literatures [11–16] on 7 case-control studies were harvested in our meta-analysis (Table 1). Furthermore, we performed a case-control study in Han Chinese population, and it was later included in our meta-analysis.

Genotype distribution of rs2228671 in our case-control study met HWE for both CHD cases and non-CHD controls (P > 0.05), indicating that our case-control study had a well-characterized random sampling. Our case-control study suggested that LDLR rs2228671-T allele was rare in Chinese population (cases: 2%; controls: 1%), and this agrees with the frequency in HapMap Chinese Han in Beijing (CHB) population (0–2%). No significant difference in the genotype distribution between CHD cases and non-CHD controls are revealed in all samples (P > 0.05; Table 2) and in the subgroup analysis by gender (P > 0.05; Table 2). In summary, our case-control study showed that there was no association between LDLR rs2228671 and CHD in Chinese. However, significant association was found between LDLR rs2228671 and CHD in European population (χ² = 20.59, P < 0.0001 by genotype; χ² = 20.26, OR = 1.180, 95% CI = 1.098–1.269, P < 0.0001 by allele; Table 3). Using the fixed-effect method, our meta-analysis contained 7,588 CHD patients and 9,711 controls from German, British, Italian, and Chinese populations. As shown in Figure 2, significant association was observed between rs2228671 and CHD (P = 0.0005, OR = 0.83, and 95% CI = 0.75–0.92). In addition, no heterogeneity among the studies was included in this meta-analysis (I² = 0%; Figure 2). Furthermore, no obvious visual evidence of publication bias in the meta-analysis was shown by funnel plot (P > 0.05; Figure 3).

4. Discussion

Aberrant LDLR level in blood can cause abnormal cholesterol metabolism [2]. As the main pathogenic gene of FH, LDLR gene is associated with multiple vascular diseases [15, 16, 27]. Polymorphisms of LDLR gene were associated with type 2 diabetes [28] and hypertension [29] that also related to CHD. Recently, a handful of LDLR polymorphisms have been studied in CHD, including those (rs14158, rs3826810, rs1433099, rs2738464, rs2738465, and rs2738466) in the 3′-untranslated region (3′-UTR) and rs2228671 in second exon [30–32]. SNPs in first intron (rs6511720) and 5′ flanking region (rs17248720) of LDLR gene were closely related to both LDL-C and CHD [33, 34]. Rs1433099 and rs2738465 in the 3′-UTR of LDLR were reported to be associated with baseline lipids in American population [32]. The T allele of rs2228671 polymorphism was associated with higher FVIII:c levels. In addition, LDLR rs2228671 may be regulated FVIII:c levels.
and associated with the independence risk factor (plasma lipids) of CHD [16].

Our meta-analysis among 17299 individuals showed that rs2228671-T allele reduced the risk of coronary heart disease in the combined samples from German, British, Italian, and Chinese populations (OR = 0.83, P = 0.0005). Furthermore, rs2228671-T allele frequencies in the meta-analysis among German, British, and Italian populations were 7–12.2% that is similar to 10% in HapMap CEU population. However, rs2228671-T allele frequency is 0% in HapMap CHB population and 0.9% in the controls of our study. Due to the rare allele of LDLR rs2228671 in our samples, the power of our case-control study was only 5.1%, in contrast of 100% in the present meta-analysis. This suggests that a lack of association in our case-control study may largely be explained by the insufficient power for this rare polymorphism and the small sample size. Future investigation on other common LDLR polymorphisms is worth being performed in a large Chinese cohort.

Human LDLR is about 43 kb in length and has 1367 active polymorphism. As shown in our study, the allele frequency of rs1122608-T is much lower than those in the European studies; suggesting a cross-population comparison of this polymorphism may help one understand the role of LDLR in different ethnic population. Meanwhile, the previous tested LDLR rs1122608 polymorphism did not yield a significant result (P = 0.148) [35], in contrast to a significant result of rs2228671 in the current study (P = 0.0005). This suggests rs2228671 and rs1122608 might exert different contributions to the risk of CHD.

There were several limitations in our study as follows. Firstly, most of the involved individuals in our meta-analysis were Europeans; thus our result might not be applied to other populations such as Chinese. Secondly, although we had no evidence of the publication bias in our meta-analysis, we cannot exclude the possibility of existing potential bias upon reporting the studies without significant association results. Last but not least, the power of our case-control study in
Chinese is only 5.1%. The negative result of rs2228671 might not represent for other variants of LDLR gene in Chinese population.

In conclusion, the meta-analysis demonstrated that the LDLR rs2228671-T allele is a protective factor of CHD in Europeans. However, the case-control study showed no significant association of LDLR rs2228671 with CHD in Han Chinese population.

Conflict of Interests

The authors declare no conflict of interests.

Authors’ Contribution

Huadan Ye, Qianlei Zhao and Yi Huang are co-first authors of this work.

Acknowledgments

The research was supported by the grants from National Natural Science Foundation of China (31100919 and 81371469), Natural Science Foundation of Zhejiang Province (LR13H020003), K. C. Wong Magna Fund in Ningbo University, Natural Science Foundation of Ningbo City (2011A610037), and Ningbo Social Development Research Projects (2012C50032).

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