Original Article

Relationships between genetic polymorphisms of E670G in PCSK9 gene and coronary artery disease: a meta-analysis

Dilare Adi1,2*, Xiang Xie1,2*, Fen Liu2, Yi-Tong Ma1,2, Mayila Abudoukelimu1,2, Yun Wu3, Yong An1, Yi-Ning Yang1,2, Xiao-Mei Li1,2, Zhen-Yan Fu1,2, Yong-Tao Wang1,2, Bang-Dang Chen2

1Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, P. R. China; 2Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi 830054, P. R. China; 3Department of General Medicine, First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, P. R. China. *Equal contributors.

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Abstract: Objective: Proprotein convertase subtilisin-like kexin type 9 (PCSK9) gene E670G Polymorphism has been reported to be associated with coronary artery disease (CAD) and risk factors. However, the results remain controversial. We sought to perform a meta-analysis to investigate the relationships between genetic polymorphisms of E670G in PCSK9 gene and the risk of CAD. Methods: Literature searches were performed to identify all published relevant case-control studies without any language restrictions. Meta-analysis was conducted using the Review Manager software (version 5.2). Heterogeneity was investigated and measured using Cochran’s Q-statistic and the inconsistency index (I²) test; Crude odds ratios (OR) with their corresponding 95% confidence interval (CI) were calculated. Results: A total of 5 case-control studies among 871 patients with CAD and 1144 control subjects were included in the meta-analysis. we found a correlation between PCSK9 genetic polymorphisms and increased risk for CAD under all of the genetic model (allele model: OR: 1.56, 95% CI: 1.21-2.01, P < 0.001; dominant model: OR: 1.46, 95% CI: 1.14-1.88, P = 0.003; recessive model: OR: 3.46, 95% CI: 1.19-10.10, P = 0.02; homozygous model: OR: 3.89, 95% CI: 1.35-11.20, P = 0.01; Heterozygous model: OR: 1.43, 95% CI: 1.08-1.92, P = 0.01; respectively). Conclusion: The results of the meta-analysis indicated that genetic polymorphism of E670G in PCSK9 gene might be involved in pathogenesis of CAD; the 670G carriers may be closely related to the risk of CAD.

Keywords: PCSK9, coronary artery disease, polymorphisms, meta-analysis

Introduction

Coronary artery disease (CAD) is the leading cause of morbidity, mortality and disability world-wide. Prevalence and number of death resulting from CAD have been predicted to increase rapidly at least until 2030 [1, 2]. CAD is a multi-factorial disorder that results from interaction of both genetic and environmental risk factors. Genetic factors account for 40%-60% in the occurrence and development of CAD [3]. Clinical and epidemiological studies have suggested that, elevated plasma level of low-density lipoprotein cholesterol (LDL-C) among lipid profiles is a primary and independent risk factor for the incidence of CAD and it has been estimated that each 1% decrease in LDL-C concentrations reduces the risk of coronary heart disease by 1% [4, 5].

Plasma concentrations of LDL-C are the major cholesterol-carrying lipoprotein and determined primarily by the activity of cell-surface LDL receptor (LDLR) in the liver. Proprotein convertase subtilisin-like kexin type 9 (PCSK9) is a newly discovered serine protease, belonging to the mammalian serine proprotein convertase (PC) family. PCSK9 was formerly named neural apoptosis regulated convertase 1 (NARC-1), not only playing major roles in regulating LDL-C homeostasis by mediating LDLR degradation through a post-transcriptional mechanism [6-9] but also being responsible for the proteolytic maturation of secretory proteins including neu-
ropeptides, prohormones, cytokines, growth factors and other cell surface proteins [10, 11].

In 2003, Abifadel and colleges [12] have identified the third gene which is implicated in Autosomal Dominant Hypercholesterolemia (ADH), named PCSK9, mapped on the short arm of chromosome 1 and localized to band p32.3. PCSK9 gene encompasses 12 exons and encodes a 692 amino acid glycoprotein. Since its first identification, accumulated evidence from different study groups has suggested that genetic variation of PCSK9 is associated with CAD, ischemic stroke and could significantly affect plasma LDL-C levels in different study populations [13-17]. Beyond that, it also could affect the Hypocholesterolemia patient’s response to Statin Therapy [18, 19]. However, relationship between genetic polymorphism of the E679G in PCSK9 gene with CAD and risk factors remains controversial. The E670G is located in the cysteine-rich C-terminal domain within exon 12 and E670G variant was found to be an independent determinant of plasma LDL-C levels and of the severity of coronary atherosclerosis in several studies [14, 15], whereas other studies demonstrated no association of E670G variant with CAD [17, 20, 21]. Hence, the current meta-analysis was conducted on all eligible case-control studies with the aim to evaluate the contribution of the PCSK9 genetic polymorphisms to CAD and to quantify the heterogeneity and potential publication bias among the involved studies.

Method

Search strategy

Literature searches were performed to identify all relevant and published case-control studies focused on the relation of polymorphisms of PCSK9 gene with CAD. Without any language restrictions we searched electronic databases (Cochrane Library Database, PubMed, MEDLIN and the Chinese Biomedical Database) since January 1980 to December 2014, using terms in conjunction with highly sensitive search strategy: [“PCSK9 gene” or “subtilisin-like kexin type 9” or “NARC-1” or “neural apoptosis regulated convertase 1”] AND [“genetic polymorphism” or “single nucleotide polymorphism” or “polymorphism” or “variation” or “SNP” or “mutation”] and [“cardiovascular disease” or “coronary artery disease” or “coronary heart disease” or “myocardial infarction”]. We also performed hand research and the reference lists of all the retrieved articles were screened.

Eligibility criteria

Criteria for inclusion were as follows: (1) a validated diagnosis of CAD. (2) Clinical case-control study focused on genetic polymorphisms of PCSK9 with CAD. (3) Genotype frequencies of healthy controls following the Hardy-Weinberg equilibrium (HWE). (4) The frequency of genotype distributions and allele frequency being available in all searched studies. Studies without any of the above inclusion criteria were excluded.

Study selection and quality assessment

Literature search, study selection and data evaluation (according to the Jadad scoring method) were completed by two investigators (Dilare Adi and Xiang Xie). Disagreements were resolved by discussion or referred to a third
investigator (Fen Liu). Complete consensus among authors on the final results was achieved. All studies included in this meta-analysis had to fulfill the eligibility criteria mentioned above. Following information was extracted from all included studies, including names of the first authors, year of publication, geographical locations, and the sources of the subjects, sample sizes and genotype frequencies.

**Statistical analysis**

All statistical analyses were performed using the Review Manager software (version 5.2, Cochrane Collaboration, Oxford, United Kingdom). Deviation from the Hardy-Weinberg equilibrium (HWE) of SNP in healthy control groups was tested by the χ² analysis. Heterogeneity was investigated and measured using Cochran’s Q-statistic and inconsistency index (I²) test, the P < 0.05 or I² > 50% indicated a substantial heterogeneity among the all included studies. The OR with 95% CI values was used to estimate the strength of association between genetic polymorphisms of PCSK9 with CAD. The combined ORs and the corresponding 95% CIs were calculated by applying either the fixed-effect or random-effect method. The Z test was used to determine the pooled OR and P < 0.05 was considered statistically significant.

**Results**

**Study selection**

Based on our search criteria, we identified 303 potentially relevant articles. By scanning the titles and the abstracts we excluded 250 articles. 53 peer-reviewed articles were retrieved for full text evaluation, 48 studies were excluded for the following reasons: 8 were not involved in polymorphisms of E679G in PCSK9 gene, 5 not case-control studies, 1 lacking any of our requisite data and 34 not related to CAD or myocardial infarction as given in Figure 1. Finally, 5 case-control studies with a total of 871 CAD patients and 1144 controls fulfilling all inclusion criteria were included in this meta-analysis. The characteristics of 5 studies [15, 19, 22-24] included in the meta-analysis are summarized in Table 1.

**Quantitative synthesis**

Our meta-analysis of the 5 eligible studies suggested that genetic variation E670G in the PCSK9 gene is associated with CAD. The genotype distribution of the E679G is in good agreement with predicted Hardy-Weinberg equilibrium values in all included studies (data not shown). Table 2 and Figure 2 showed that PCSK9 genetic polymorphisms might be correlated with the risk for CAD under all the genetic

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**Table 1.** Baseline characteristics of the all included studies in the Meta-analysis

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Population</th>
<th>Sample size</th>
<th>Genotypes distribution (case/control)</th>
<th>Sex (male)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung-An Hsu</td>
<td>2008</td>
<td>Taiwan</td>
<td>202</td>
<td>614</td>
<td>182/541</td>
<td>55.6±10.5</td>
</tr>
<tr>
<td>Qiu Yu</td>
<td>2011</td>
<td>Chinese Han</td>
<td>100</td>
<td>100</td>
<td>78/90</td>
<td>45.9±10.4</td>
</tr>
<tr>
<td>Meng yanhui</td>
<td>2011</td>
<td>Chinese Han</td>
<td>165</td>
<td>180</td>
<td>146/166</td>
<td>66.49±9.92</td>
</tr>
<tr>
<td>ZENG Jian</td>
<td>2011</td>
<td>Chinese Han</td>
<td>212</td>
<td>184</td>
<td>167/165</td>
<td>65.9±10.2</td>
</tr>
<tr>
<td>Afef Slimani</td>
<td>2014</td>
<td>Monastir</td>
<td>192</td>
<td>66</td>
<td>148/57</td>
<td>61 (55-65)</td>
</tr>
</tbody>
</table>

**Table 2.** Meta-analysis of the relationship between genetic polymorphisms of PCSK9 gene with coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>G vs. case'2 (Allele model)</th>
<th>AG + GG vs. Total (Dominant model)</th>
<th>GG vs. Total (Recessive model)</th>
<th>GG vs. AA + GG (Homozygous model)</th>
<th>AG vs. AA + AG (Heterozygous model)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI  P</td>
<td>OR 95% CI  P</td>
<td>OR 95% CI  P</td>
<td>OR 95% CI  P</td>
<td>OR 95% CI  P</td>
</tr>
<tr>
<td>Lung-An Hsu</td>
<td>0.82 0.51-1.33 P</td>
<td>0.83 0.52-1.33 P</td>
<td>1.01 0.04-24.69 P</td>
<td>0.99 0.04-24.39 P</td>
<td>0.83 0.49-1.39 P</td>
</tr>
<tr>
<td>ZENG Jian</td>
<td>2.15 1.32-3.50</td>
<td>1.48 0.77-2.86</td>
<td>3.63 14.24 P</td>
<td>3.46 0.71-16.89 P</td>
<td>2.18 0.92-5.17 P</td>
</tr>
<tr>
<td>Meng yanhui</td>
<td>1.48 0.75-2.90</td>
<td>2.2 1.10-4.40</td>
<td>-</td>
<td>-</td>
<td>1.54 0.75-3.19 P</td>
</tr>
<tr>
<td>Qiu Yu</td>
<td>2.45 1.25-4.81</td>
<td>2.06 1.25-3.38</td>
<td>5.59 42.04 P</td>
<td>5.77 0.66-50.44 P</td>
<td>2.21 1.20-4.07 P</td>
</tr>
<tr>
<td>Afef Slimani</td>
<td>1.95 0.99-3.85</td>
<td>1.68 0.87-3.25</td>
<td>5.21 0.30-89.95</td>
<td>5.81 0.33-103.35 P</td>
<td>1.58 0.72-3.49 P</td>
</tr>
<tr>
<td>Total</td>
<td>1.56 1.21-2.01 &lt; 0.001</td>
<td>1.46 1.14-1.88 0.003</td>
<td>3.46 1.19-10.10 0.02</td>
<td>3.89 1.35-11.20 0.01</td>
<td>1.43 1.08-1.92 0.01</td>
</tr>
</tbody>
</table>
Figure 2. Forest plot for PCSK9 gene E670G polymorphism and CAD risk in different genetic models: A. (Allele model: G vs. case’2); B. (Dominant model: AG + GG vs. total); C. (Recessive: GG vs. total); D. (Homozygous model: GG vs. AA + GG); E. (Heterozygous model: AG vs. AA + AG).
Figure 3. Funnel plots for PCSK9 gene E670G polymorphism and CAD risk in different genetic models: A. (Allele model: G vs. case); B. (Dominant model: AG + GG vs. total); C. (Recessive: GG vs. total); D. (Homozygous model: GG vs. AA + GG); E. (Heterozygous model: AG vs. AA + AG).
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model (allele model: OR: 1.56, 95% CI: 1.21-2.01, P < 0.001; dominant model: OR: 1.46, 95% CI: 1.14-1.88, P = 0.003; recessive model: OR: 3.46, 95% CI: 1.19-10.10, P = 0.02; homozygous model: OR: 3.89, 95% CI: 1.35-11.20, P = 0.01; Heterozygous model: OR: 1.43, 95% CI: 1.08-1.92, P = 0.01; respectively). Due to existence of heterogeneity, random effect model was used in allele model and dominant model in our Meta analysis. No evidence of asymmetry was observed in the funnel plots as shown in Figure 3.

Discussion

We conducted a meta-analysis on all published eligible studies via collecting summary statistics on the association of E679G polymorphism in PCSK9 gene using different genetic models. The results of current meta-analysis showed that E679G mutation of PCSK9 gene was associated with an increased CAD risk. To the best of our knowledge, this was the first Meta analysis to investigate association of E679G polymorphism in PCSK9 gene with CAD.

PCSK9 is the newest member of proprotein convertase family with a molecular weight of 72 kDa that consists of a prodomain (PD), a catalytic domain, a cysteine and histidine rich C-terminal domain (CHRD) [25]. PCSK9 is identified as the third locus implicated in autosomal dominant hypercholesterolemia (ADH). Studies have indicated that genetic polymorphisms of PCSK9 were associated with the pathogenesis and progression of some diseases such as CAD and stroke, and could also significantly affect plasma LDL-c. Most importantly, PCSK9 has emerged as a promising treatment target to lower cholesterol, especially LDL-C and thus to cardiovascular risk profiles. Since deletion of cysteine-rich C-terminal domain E670G in animal models was reported to cause accumulation of processed PCSK9 [26], a bunch of studies demonstrated that by virtue of major regulator and inhibitor of LDLR at a post-transcriptional level, PCSK9 E670G variant was found to be an independent determinant of plasma LDL-c and to be related to the severity of coronary atherosclerosis in different populations. Suet Chen et al found that in African-Americans and Whites a common haplotype containing E670G variant was associated with severity of coronary atherosclerosis, and plasma LDL-c levels showed significant differences between AA and AG genotypes [27], supported by the study conducted by Evans and Beil reporting that E670G polymorphism in European males was associated with hypercholesterolemia. That is, males with GG or AG genotypes had higher plasma LDL-c, than did those with AA genotypes [16]. Meanwhile, Afef Slimani et al found that plasma TC and LDL-c were significantly higher in CAD patients carrying 670 G genotype than in controls, and the risk and severity of CAD were significantly higher in CAD subgroup who presented stenosis ≥ 50% in two or three major coronary arteries than in non-CAD subgroup carrying 670 G genotypes [15], which was consistent with the results reported by Meng Yan-hui et al who found that the risk and severity were significantly increased in CAD patients of 670 G carriers, compared to controls [23].

In the present meta-analysis we collected aggregated data from 5 studies with a total of 2015 (871 cases and 1148 controls) subjects, analyzed using standardized models and revealed a positive correlation between PCSK9 genetic polymorphisms and increased risk for CAD under all the genetic models (allele model: OR: 1.56, 95% CI: 1.21-2.01, P < 0.001; dominant model: OR: 1.46, 95% CI: 1.14-1.88, P = 0.003; recessive model: OR: 3.46, 95% CI: 1.19-10.10, P = 0.02; homozygous model: OR: 3.89, 95% CI: 1.35-11.20, P = 0.01; Heterozygous model: OR: 1.43, 95% CI: 1.08-1.92, P = 0.01; respectively), which might be widely explained by previous findings.

Current meta-analysis, however, harbors some limitations. First, heterogeneity might exist due to diversity in study designs, ethnicity, sample sizes, genotyping methods and in risk profiles among studies. Second, publication bias might also be present since studies with negative results are harder to be accepted than are those with positive findings. Third, relatively little small sample size and the confounding risk factors for CAD in all included study vary from each other and also because of the limited publications about the relationship between polymorphisms of E679G in PCSK9 gene with CAD; we are limited to perform subgroup analysis among diverse ethnicity, implicating necessities of more studies to be conducted. Finally, lack of original data from eligible studies limited evaluation of effects of genetic-environmental interactions during CAD development and further adjustments of the results by other vari-
ables, such as gender, hypertension, Diabetes mellitus, obesity, smoking and other lifestyle factors.

Despite limitations, however, several advantages are contained in this meta-analysis. First, because PCSK9 has become the prospective target to lower LDL-c since statins used and to intervene atherosclerotic diseases, and thus current findings could provide some additional information that PCSK9 might be a possible target for medical treatment. Second, based on previous experiences, it was feasible to perform this study and substantial numbers of participants were pooled from different studies which helps us draw a more reliable conclusion.

In conclusion, a significant association that E679G genetic polymorphisms in PCSK9 might be involved in CAD pathogenesis is identified; the 670 G carriers may be closely related to the risk of CAD. However, further studies with larger sample sizes on different ethnicity using rigorous study designs, well matched case and controls (especially in risk factors status) are needed to be conducted; and there is a greater need in genetics epidemiology for quantitative systematic reviews to obtain more conclusive results.

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Disclosure of conflict of interest

None.

Address correspondence to: Yi-Tong Ma, Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, P. R. China. E-mail: myt_xj@sina.com

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