



Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T polymorphisms and risk of coronary heart disease

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Complete List of Authors:	Wu, Yihua; School of Public Health, Zhejiang University, Department of Epidemiology and Health Statistics Lin, Bin; Wenzhou Central Hospital, Department of Cardiology Huang, Yiwei; Wenzhou Central Hospital, Department of Cardiology Zhang, Mingying; Wenzhou Central Hospital, Department of Cardiology Wang, Jun; Wenzhou Central Hospital, Department of Cardiology
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4 **Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T**

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6 **polymorphisms and risk of coronary heart disease**

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9 Yihua Wu² Bin Lin^{1*}, Yiwei Huang¹, Mingying Zhang¹, Jun Wang¹, *

10
11 ¹Department of Cardiology, Wenzhou Central Hospital, Wenzhou, 325000 China;

12
13 ²Department of Epidemiology and Health Statistics, Zhejiang University School of
14
15 Public Health, Hangzhou, 310058 China;

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17
18 (Running title: ApoC3 polymorphisms and CHD risk)

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20
21 *Corresponding Author:

22
23
24 Bin Lin, MD, Department of Cardiology, Wenzhou Central Hospital, Wenzhou,
25
26 325000 China;

27
28
29 Tel: 13706670776

30
31 E-mail: d4c3b2a@sina.com

32
33
34 Yihua Wu, PhD, Department of Epidemiology and Health Statistics, Zhejiang
35
36 University School of Public Health, Hangzhou, 310058 China;

37
38
39 Tel/Fax: 86-0571-88208140

40
41
42 E-mail: georgewuer@126.com

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Abstract

Objectives: Apolipoprotein C3 (ApoC3) polymorphisms have been suggested to be associated with risk of coronary heart disease (CHD). However, the results of relevant studies were inconsistent. We aimed to systematically evaluate this issue.

Design: Pubmed and Embase databases (up to March 2013) were systematically searched to identify studies evaluating the association between ApoC3 polymorphisms and CHD risk. Two reviewers independently identified studies, extracted and analyzed data. Either a fixed- or a random-effects model was adopted to estimate overall odds ratios (ORs).

Studies reviewed: Finally, twenty-one studies comprising 15591 participants were included in this systematic review.

Results: Under dominant model, SstI polymorphism was borderline significantly associated with CHD risk (S1S2+S2S2 vs. S1S1, pooled OR=1.19, 95% CI 1.00-1.42). Subgroup analyses suggested that SstI polymorphism was significantly associated with myocardial infarction (MI) risk (pooled OR=1.42, 95% CI 1.06-1.91), and SstI polymorphism was statistically associated with CHD risk among Asian population (pooled OR=1.35, 95% CI 1.08-1.69) and in retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61). Significant association was observed between T-455C polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42). C-482T and C1100T polymorphisms were not indicated to be associated with CHD risk.

Conclusions: ApoC3 Sst I and T-455C polymorphisms might be associated with

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4 CHD risk significantly.

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6 **Key Words:** coronary heart disease, apolipoprotein C-III, genetic polymorphisms,
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8 risk factors, meta-analysis.
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10 11 12 **Article focus**

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15 The aim of this study was to systematically evaluate the association between
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17 apolipoprotein C3 polymorphisms and CHD risk.
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19 20 **Key message**

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22 This study shows that ApoC3 Sst I and T-455C polymorphisms might be associated
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24 with CHD risk significantly.
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27 28 **Strengths and limitations of the study**

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30 The present study included twenty-one studies comprising 15591 subjects and
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32 comprehensively evaluated the association between ApoC3 polymorphisms and CHD
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34 risk.
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38 Most of the included studies were from Asia, Europe and USA, so the conclusions
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40 may not be true for other ethnic groups. Besides, between-study heterogeneity could
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42 not be completely explained.
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Introduction

The progression of CHD is complicated and is influenced by multi genetic and environmental factors, and atherosclerosis of coronary artery is the basic pathogenic factor of CHD¹. Plasma lipids and lipoproteins are important risk factors for atherosclerosis, and genes involved in lipoprotein metabolism might be candidate genes for CHD susceptibility^{2 3}. Apolipoprotein C3 (ApoC3) is an essential component of circulating particles in TG-rich lipoprotein, and inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by Apolipoprotein E⁴. Therefore, high levels of ApoC3 may cause hypertriglyceridemia⁵. Previous studies supported that ApoC3 might play an important role in CHD development, and the ApoC3 concentration was also associated with CHD risk^{6 7}.

Serum ApoC3 concentration was shown to be influenced by genetic and acquired factors⁸. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism⁹⁻¹². In recent years, studies have investigated the correlation between these polymorphisms and CHD risk, while the results were inconsistent. The aim of our meta-analysis was to assess the association between ApoC3 polymorphisms and risk of CHD.

Methods

Literature search

Systematic literature searches were conducted before March 2013 in Pubmed and Embase databases without restrictions. Combination of the following terms were applied: “coronary heart disease” OR “coronary artery disease” OR “myocardial

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4 infarction” OR “acute coronary syndrome” OR “ischemic heart disease” OR
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6 “cardiovascular disease” OR “CHD” OR “CAD” OR “MI” OR “ACS” OR “IHD”;
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8 “apolipoprotein C3” OR “apolipoprotein C” OR “apolipoprotein C- III” OR
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10 “apolipoprotein C III” OR “APO C3” OR “APOC3” OR “APOC” OR “APO C III”
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12 OR “APO C- III”; “polymorphism” OR “variant” OR “SNP” OR “mutation”.
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References of relevant articles were also scanned for studies potentially missed in the primary searches. Articles published in English were retrieved. And the retrieved studies were carefully examined to exclude potential duplicates or overlapping data. This meta-analysis was designed, conducted and reported according to PRISMA statement¹³.

Selection criteria and data extraction

Studies retrieved from the initial search were then screened for eligible articles. Titles and abstracts were scanned and then full papers were reviewed. We included articles if they met all the following criteria: (1) evaluating the association between ApoC3 polymorphism and coronary heart disease; (2) odds ratio (OR) estimates and their 95% confidence intervals (95%CI) were available or could be calculated; (3) each polymorphism included in the meta-analysis should be reported by at least two studies.

Data were extracted independently by two reviewers (Bin Lin and Yiwei Huang). The following information was extracted from each study: first author, publication year, country, study design, sample size, gender distribution, mean age, phenotype (disease), genotype of cases and controls. Any discrepancy was resolved by a third

investigator.

Statistical analysis

Dominant model was applied in this study as this genetic model was most widely used in the included studies. Because some studies did not apply dominant model, we re-calculated OR values under dominant model. Either a fixed-effects model or a random-effects model was applied to pool the OR estimates of each study, according to heterogeneity across studies. The extent of heterogeneity was checked using the chi-square test and I^2 test; $p \leq 0.10$ and/or $I^2 > 50\%$ indicates significant heterogeneity. When $p > 0.10$, the fixed-effects model was applied and otherwise we used the random-effects model. Subgroup analysis was applied to explore heterogeneity. Funnel plots were constructed and Begg's and Egger's tests were used to assess publication bias, and $p \leq 0.10$ was considered to be significant. All analyses were conducted using the Stata software (version 11.0; StatCorp, College Station, TX, USA).

Results

Study selection and characteristics

A total of 1399 articles were identified by searching Pubmed and Embase databases. Among them, 1355 articles were excluded through screening titles and abstracts. The remaining 44 articles were carefully evaluated as full texts and 23 articles were excluded. The reasons for exclusion were articles not on right topic (13 papers), insufficient data (4 papers), relevant reviews (6 papers). This meta-analysis finally included 21 articles^{7 9-12 14-29}. The selection process was shown in Figure 1 while the

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3 characteristics of those studies were listed in Supplementary Table 1. Among these
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5 studies, fifteen studies assessed ApoC3 Sst I polymorphism, four studies evaluated
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7 ApoC3 T-455C polymorphism, four studies reported ApoC3 C-482T polymorphism
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9 and three investigated ApoC3 C1100T polymorphism (several studies reported more
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11 than one polymorphism).
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15 **AOPC3 polymorphisms and risk of CHD**

16 **Meta-analysis of Sst I polymorphism**

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19 A total of 15 studies with 11539 individuals assessed the association between Sst I
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21 polymorphism and CHD risk. Under dominant model (S1S2+S2S2 vs. S1S1), the
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23 pooled OR of all studies was 1.19 (95% CI 1.00-1.42) (Figure 2, Table 1), indicating a
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25 borderline significant association between Sst I polymorphism and CHD risk. There
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27 was significant heterogeneity among studies ($I^2 = 48.9\%$, $p=0.017$) (Figure 2, Table
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36 According to study characteristics, subgroup analysis was adopted, as shown in
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38 Table 2. Pooled results showed that S1S2 and S2S2 genotypes might increase risk of
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40 CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian
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42 population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence
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44 the result, Sst I polymorphism was significantly associated with CHD risk in
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46 retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective
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48 studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was
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50 observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI
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52 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35).
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Meta-analysis of T-455C polymorphism

The association between T-455C polymorphism and CHD risk was evaluated by 4 studies comprising 3378 individuals. Results indicated significant association between T-455C polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42) (Figure 3, Table 1). No significant heterogeneity among studies was indicated ($I^2 = 0\%$, $p=0.580$) (Figure 3, Table 1).

Meta-analysis of C-482T polymorphism

Four studies with 3070 individuals reported the association between C-482T polymorphism and CHD risk. There was no significant association between C-482T polymorphism and CHD risk (CT+TT vs. CC, pooled OR=1.06, 95% CI 0.92-1.22) (Figure 4, Table 1). No significant heterogeneity was observed ($I^2 = 0\%$, $p=0.788$) (Figure 4, Table 1).

Meta-analysis of C1100T polymorphism

Three studies comprising 4662 participants evaluated the association between C1100T polymorphism and CHD risk. No significant association was found (CT+TT vs. CC, pooled OR=1.06, 95% CI 0.89-1.27) and no significant heterogeneity was observed ($I^2 = 46.7\%$, $p=0.153$) (Figure 5, Table 1).

Publication bias

Begg's and Egger's tests suggested that no publication bias was found in our meta-analyses.

Discussion

ApoC3 is a glycoprotein synthesized mainly in the liver and the intestinal, and

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4 plays an essential role in regulating the serum triglyceride levels. Besides, it can
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6 strongly regulate the levels of VLDL and small dense LDL which potentially
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8 improves atherosclerosis³⁰. Clinical research found that ApoC3 levels were predictor
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10 of risk for development of CHD³¹⁻³³. In the present study, twenty-one studies were
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12 included and four polymorphisms of ApoC3 were evaluated, including C-482T,
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14 T-455C, Sst I and C1100T polymorphism. T-455C polymorphism was suggested to be
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16 significantly associated with CHD risk, and 'C' allele increased CHD risk by 22
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18 percent (CT+TT vs. CC, pooled OR=1.22, 95% CI 1.06-1.42). A borderline
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20 significant association was observed between Sst I polymorphism and CHD risk.
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22 While no evidence suggested significant association between C-482T and C1100T
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24 polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism,
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26 and we found that Sst I polymorphism was significantly associated with MI risk.
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28 Besides, Sst I polymorphism was significantly associated with CHD risk in Asian
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30 population but not in Caucasian population, indicating that the effect of Sst I
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32 polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was
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34 indicated to be significantly associated with CHD risk but this association was not
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36 confirmed in prospective studies. So, the association between Sst I polymorphism and
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38 CHD risk should be interpreted cautiously.
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49 The mechanism of Sst I polymorphism in CHD susceptibility may be multiple.
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51 Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is
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53 possible that this polymorphism is in linkage disequilibrium with other functional
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55 polymorphism in the nearby region, such as T-455C polymorphism¹⁰. Sst I
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4 polymorphism might alter plasma lipid concentrations. Several studies showed S2
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6 carriers have higher plasma total cholesterol, TG and LDL-C levels^{12 34 35}, though
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8 other studies did not demonstrate significant difference^{10 19 36}. Besides, it has been
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10 shown that S2 allele might significantly influence dyslipidemic state and
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12 atherosclerosis severity when patients changed their diet from saturated fatty acids to
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14 olive oil³⁷. So, Sst I polymorphism plays an important role in modulating lipid levels
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16 response to dietary changes.
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21 T-455C and C-482T polymorphisms both located in the 5' promoter region and
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23 were in strong linkage disequilibrium with each other. These two polymorphisms
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25 have been studied extensively because they could alter the nuclear transcript factors
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27 which mediate the insulin response. Significant association between T-455C
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29 polymorphism and risk of CHD was found under dominant model. For C-482T
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31 polymorphism, no significant association with CHD risk was found.
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37 Different mechanisms could be linked to this finding. In previous works, T-455C
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39 polymorphism was associated with increased TG and ApoC3 levels^{23 38}. Also, T-455C
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41 polymorphism was demonstrated to be significantly interacted with metabolic
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43 syndrome³⁸. Another study showed T-455C polymorphism could interfere with n-3
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45 polyunsaturated fatty acids on ApoC3 concentrations⁷. They found that CC
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47 homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3
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49 polyunsaturated fatty acids⁷.
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54 The present meta-analysis has several strengths. First, our study included
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56 twenty-one studies comprising 15591 subjects, which was sufficient to allow adequate
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4 statistical power to calculate the results. Second, we comprehensively evaluated the
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6 association between ApoC3 polymorphisms and CHD risk, and a total of four
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8 polymorphisms of ApoC3 were assessed. Besides, no publication bias was observed,
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10 indicating the pooled results might be unbiased.
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14 The current analysis also has several limitations. First, most of the included studies
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16 investigated Asian or Caucasian population, so the conclusions may not be true for
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18 other ethnic groups. Second, significant heterogeneity was found and could not be
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20 completely explained when assess some polymorphisms. Finally, only articles
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22 published in English were included.
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26 In summary, ApoC3 Sst I and T-455C polymorphisms might be associated with
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28 CHD risk, while no evidence suggested significant association between C-482T and
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30 C1100T polymorphisms and CHD risk.
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40 Wu contributed to conception, design of the study, and editing the manuscript; Drs.
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42 Yiwei Huang and Mingying Zhang contributed to data acquisition, analysis, and
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44 interpretation of the data. Dr. Jun Wang contributed to statistical analysis and editing
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51 **Data sharing statement:** No additional data are available.

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54 We declare that we have no conflict of interest.
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Contributorship Statement:

The Corresponding Authors (Drs. Bin Lin and Yihua Wu) have the right to grant on behalf of all authors and does grant on behalf of all authors. Drs. Bin Lin and Yihua Wu contributed to conception, design of the study, and editing the manuscript; Drs. Yiwei Huang and Mingying Zhang contributed to data acquisition, analysis, and interpretation of the data. Dr. Jun Wang contributed to statistical analysis and editing the manuscript.

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Table 1 Meta-analysis results of Sst I, T-455C, C-482T, C1100T polymorphisms

Polymorphism	No. of studies	No. of participants	Comparison	OR (95% CI)	Heterogeneity	
					I ² (%)	P
Sst I	15	11539	S1S2+S2S2 vs. S1S1	1.19 (1.00-1.42)	48.9	0.017
T-455C	4	3378	TC+CC vs. TT	1.22 (1.06-1.42)	0	0.580
C-482T	4	3070	CT+TT vs. CC	1.06 (0.92-1.22)	0	0.788
C1100T	3	4662	CT+TT vs. CC	1.06 (0.89-1.27)	46.7	0.153

Table 2 Subgroup analysis of Sst I polymorphism

Groups	OR (95% CI)	Heterogeneity		
		I ² (%)	P	
Ethnicity	Caucasian	1.14 (0.92-1.41)	54.2	0.013
	Asian	1.35 (1.08-1.69)	0	0.427
Study design	Retrospective	1.30 (1.04-1.61)	39.0	0.098
	Prospective	0.98 (0.75-1.28)	52.3	0.098
Phenotype	CHD	1.09 (0.87-1.35)	46.0	0.047
	MI	1.42 (1.06-1.91)	52.3	0.099

Figure legends

Figure 1. Flow diagram of study selection process.

Figure 2. Association between Sst I polymorphism and CHD risk.

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Figure 3. Association between T-455C polymorphism and CHD risk.

Figure 4. Association between C-482T polymorphism and CHD risk.

Figure 5. Association between C1100T polymorphism and CHD risk.

Supplementary file.

Supplementary Table 1 Characteristics of the included studies

Checklist PRISMA checklist

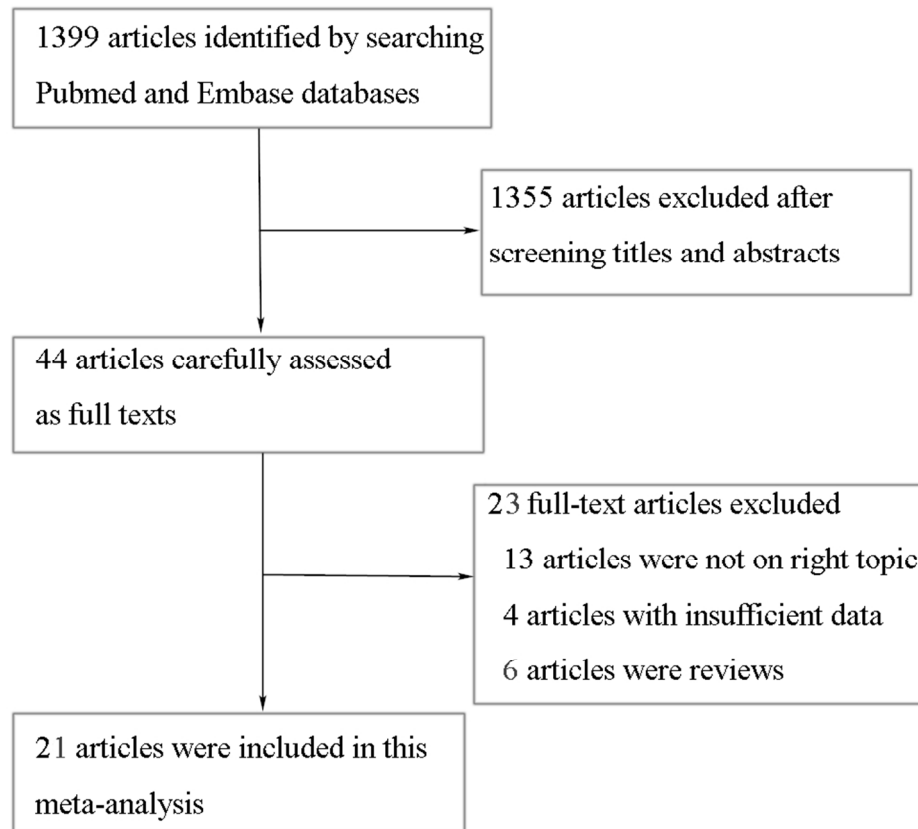


Figure 1. Flow diagram of study selection process.
88x88mm (300 x 300 DPI)

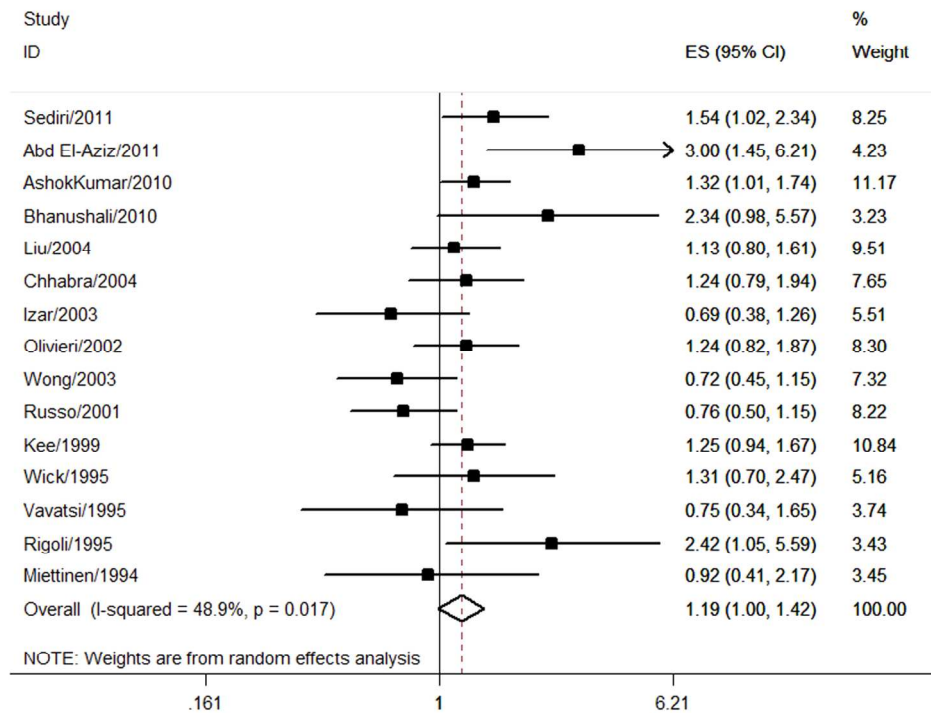


Figure 2. Association between Sst I polymorphism and CHD risk.
127x102mm (300 x 300 DPI)

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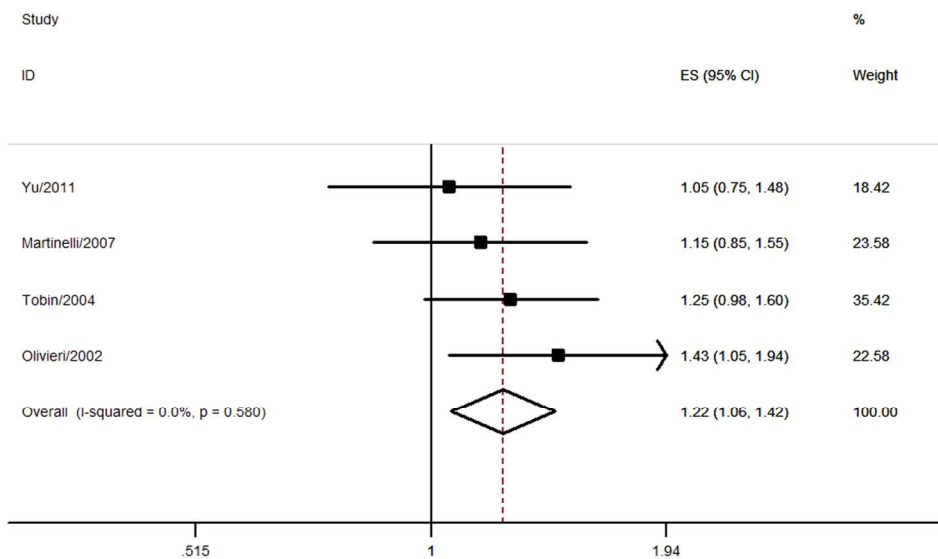


Figure 3. Association between T-455C polymorphism and CHD risk.
127x84mm (300 x 300 DPI)

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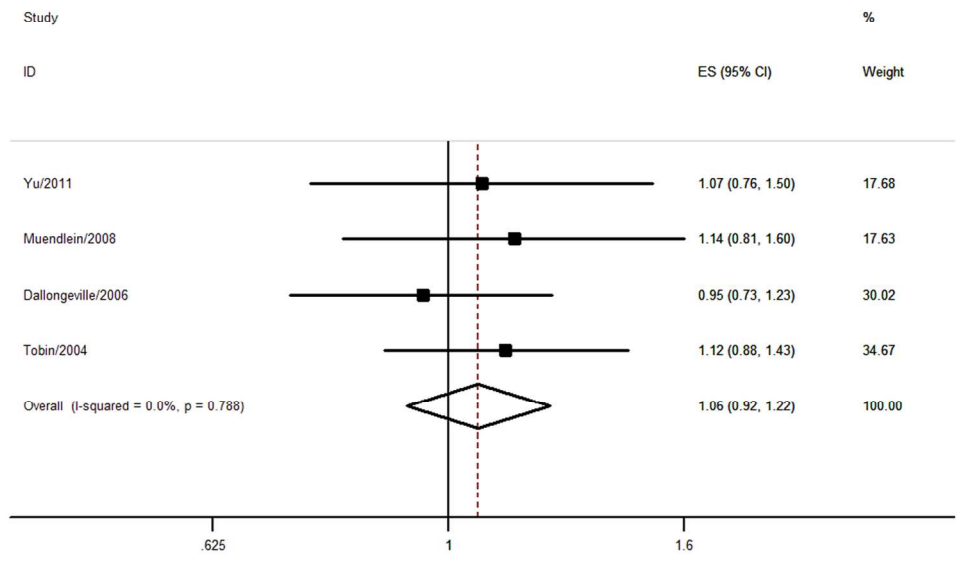


Figure 4. Association between C-482T polymorphism and CHD risk.
127x84mm (300 x 300 DPI)

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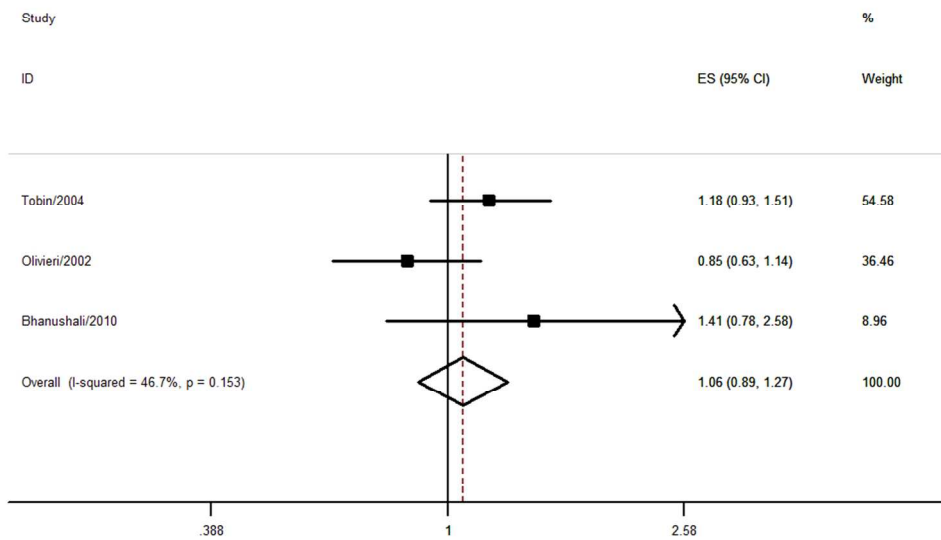


Figure 5. Association between C1100T polymorphism and CHD risk.
127x82mm (300 x 300 DPI)

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Supplementary Table 1 Characteristics of the included studies

Study and Publication year	Country	Study design	Sample size (cases/controls)	Sex (M/F)	Mean Age (years)	Pheno type	Mutation or polymorphisms	Cases			Controls			HWE
								aa	ab*	bb	aa	ab	bb	
Yu/2011 ⁹	China	Case-control	611(286/325)	Cases: 214/72	Cases: 56.3	CHD	T-455C	90	13	47	11	15	54	Yes
				Controls: 172/153	Controls: 55.79			1		2	7			
Sediri/2011 ¹⁰	Tunisia	Case-control	687(326/361)	Cases: 326/0	Cases: 53.8	MI	Sst I	26	53	7	31	45	1	Yes
				Controls: 361/0	Controls: 51.1			6		5				
Abd El-Aziz/2011 ¹²	Egypt	Case-control	300(200/100)	Cases: 67/33	Cases: 52.9	AMI	Sst I	15	42	8	70	10	20	Yes
				Controls: 32/18	Controls: 50.7			0						
AshokKumar/2010 ¹⁴	India	Case-control	832(416/416)	Cases: 322/94	Cases: 53.23	CHD	Sst I	18	19	34	21	17	22	Yes
				Controls: 315/101	Controls: 53.59			9	3		8	6		
Bhanushali/	India	Case-control	240(50/1	Cases:	Cases:	CHD	Sst I and	-	-	-	-	-	-	-

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2010 ¹¹		ol	90)	82/8	47		C1100T								
				Controls:	Controls:										
				146/44	48										
Muendlein/2008 ¹⁵	Austria	Cross-section	557(332/225)	Cases: 264/68	Cases: 62.5	CHD	C-482T	16	14	27	11	87	21	Yes	
				Controls: 123/102	Controls: 61.5			2	3		7				
Martinelli/2007 ¹⁶	Italy	Case-control	913(669/244)	Cases: 544/125	Cases: 60.7	CHD	T-455C	24	30	12	97	11	29	Yes	
				Controls: 168/76	Controls: 58.7			4	0	5		8			
Dallongeville/2006 ¹⁷	France	Case-control	917(442/475)	Cases: 442/0	Cases: 35-64	CHD and MI	C-482T	23	15	35	25	18	31	Yes	
				Controls: 475/0	Controls: 35-64			7	5		5	5			
Olivieri/2005 ⁷	Italy	Cross-section	848(590/258)	Cases: 486/104	Cases: 60.5	CHD	T-455C	21	27	10	11	11	24	Yes	
				Controls: 165/93	Controls: 58.3			2	1	7	6	8			
Tobin/2004 ¹⁸	UK	Case-control	1054(549/505)	Cases: 372/177	Cases: 61.9	MI	C-482T	29	23	23	28	19	29	Yes	
				Controls: 313/192	Controls: 58.6			1	3		3	3			
							T-455C	21	28	52	21	22	62	Yes	
								1	4		4	9			
							C1100T	29	20	40	29	17	37	Yes	

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Liu/2004 ¹⁹	USA	Nested case-control	758(385/373)	Cases: 385/0 Controls: 373/0	Cases: 60 Controls: 59	MI	Sst I	29 5	77	6	29 7	60	4	Yes
Chhabra/2004 ²⁰	India	Case-control	309(158/151)	Cases: 139/19 Controls: 139/12	Cases: 53.25 Controls: 52.45	CHD	Sst I	66	76	16	71	66	14	Yes
Wong/2003 ²¹	UK	Cohort	2808(187/2621)	Cases: 187/0 Controls: 2621/0	Cases: 56.67 Controls: 56.01	CHD	C1100T C-428T and Sst I	-	-	-	-	-	-	-
Izar/2003 ²²	Brazil	Case-control	224(112/112)	Cases: 65/47 Controls: 66/46	Cases: 46 Controls: 45	CHD	Sst I	81	23	3	71	32	1	Yes
Olivieri/2002 ²³	Italy	Cross-section	800(549/251)	Cases: 449/100 Controls: 168/83	Cases: 60.4 Controls: 57.6	CHD	T-455C C1100T Sst I	19 4	25 3	10 2	11 0	11 8	23	Yes
								29	20	46	12	10	17	Yes
								8	5		6	8		
								45	97	0	21	37	0	Yes
								2			4			
Russo/2001 ²⁴	USA	Cohort	2485(202)	Cases: -	-	CHD	Sst I	-	-	-	-	-	-	-

4			/2283)	146/56											
				Controls:											
				1133/1150											
Kee/1999 ²⁹	UK	Cohort	1375(761/614)	Cases: 761/0	-	MI	Sst I	50	11	1	64	11	3	Yes	
				Controls: 614/0				1	2		5	3			
Wick/1995 ²⁵	Germany	Case-contr ol	313(212/101)	-	-	CHD	Sst I	17	42	0	85	16	0	Yes	
								0							
Vavatsi/1995 ²⁶	Greece	Case-contr ol	149(95/54)	Cases: 85/10	Cases: 51	CHD	Sst I	69	20	0	36	12	2	Yes	
				Controls: 46/9	Controls: 50										
Rigoli/1995 ²⁷	Italy	Case-contr ol	124(62/62)	Cases: 43/19	Cases: 58.2	CHD	Sst I	41	21	0	52	10	0	Yes	
				Controls: 42/20	Controls: 57.6										
Miettinen/1994 ²⁸	Finland	Case-contr ol	132(82/50)	Cases: 78/4	Cases: 40.8	CHD	Sst I	62	19	1	37	12	1	Yes	
				Controls: 42/8	Controls: 38.7										

MI: myocardial infarction; AMI: acute myocardial infarction; CHD: coronary heart disease.
 -: not reported.

Checklist PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			

Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6-7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7-8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7-8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7-8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7-8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7-8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097



Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T polymorphisms and risk of coronary heart disease

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine, Genetics and genomics
Keywords:	Coronary heart disease < CARDIOLOGY, GENETICS, Cardiology < INTERNAL MEDICINE

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Manuscripts

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4 **Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T**

5
6 **polymorphisms and risk of coronary heart disease**

7
8 Bin Lin^{1*}, Yiwei Huang¹, Mingying Zhang¹, Jun Wang¹, Yihua Wu^{2*}

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10
11 ¹Department of Cardiology, Wenzhou Central Hospital, Wenzhou, 325000 China;

12
13 ²Department of Epidemiology and Health Statistics, Zhejiang University School of
14
15 Public Health, Hangzhou, 310058 China;

16
17
18 (Running title: ApoC3 polymorphisms and CHD risk)

19
20
21 *Corresponding Authors:

22
23
24 Bin Lin, MD, Department of Cardiology, Wenzhou Central Hospital, Wenzhou,
25
26 325000 China;

27
28
29 Tel: 13706670776

30
31 E-mail: d4c3b2a@sina.com

32
33
34 Yihua Wu, PhD, Department of Epidemiology and Health Statistics, Zhejiang
35
36 University School of Public Health, Hangzhou, 310058 China;

37
38
39 Tel/Fax: 86-0571-88208140

40
41
42 E-mail: georgewuer@126.com

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46 We declare that we have no conflict of interest.
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54 **Key Words:** coronary heart disease, apolipoprotein C-III, genetic polymorphisms,
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56 risk factors, meta-analysis
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Abstract

Objectives: Apolipoprotein C3 (ApoC3) polymorphisms have been suggested to be associated with risk of coronary heart disease (CHD). However, the results of relevant studies were inconsistent. We aimed to systematically evaluate this issue.

Design: Pubmed, Embase and Cochrane library databases (up to March 2013) were systematically searched to identify studies evaluating the association between ApoC3 polymorphisms and CHD risk. Two reviewers independently identified studies, extracted and analyzed data. Either a fixed- or a random-effects model was adopted to estimate overall odds ratios (ORs).

Studies reviewed: Finally, twenty studies comprising 15591 participants were included in this systematic review. Fifteen studies with 11539 individuals were included in the meta-analysis of Sst I polymorphism, four studies comprising 3378 individuals assessed T-455C polymorphism, four studies with 3070 participants evaluated C-482T polymorphism and C1100T polymorphism was assessed by 3 studies comprising 4662 participants.

Results: Under dominant model, Sst I polymorphism was borderline significantly associated with CHD risk (S1S2+S2S2 vs. S1S1, pooled OR=1.19, 95% CI 1.00-1.42). Subgroup analyses suggested that Sst I polymorphism was significantly associated with myocardial infarction (MI) risk (pooled OR=1.42, 95% CI 1.06-1.91), and Sst I polymorphism was statistically associated with CHD risk among Asian population (pooled OR=1.35, 95% CI 1.08-1.69) and in retrospective studies (pooled OR=1.30,

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4 95% CI 1.04-1.61). Significant association was observed between T-455C
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6 polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42).
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9 A borderline significant association was suggested between T-455C polymorphism
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11 and MI risk (pooled OR=1.21, 95% CI 1.00-1.46). C-482T and C1100T
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13 polymorphisms were not indicated to be associated with CHD risk or MI risk.
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16 **Conclusions:** ApoC3 Sst I and T-455C polymorphisms might be associated with
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18 CHD risk.
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Article focus

The aim of this study was to systematically evaluate the association between apolipoprotein C3 polymorphisms and CHD risk.

Key message

This study shows that ApoC3 Sst I and T-455C polymorphisms might be associated with CHD risk.

Strengths and limitations of the study

The present study comprehensively evaluated the association between ApoC3 polymorphisms, including Sst I, T-455C, C-482T, C1100T and CHD risk. The methods of this study were rigorous and were based on guidelines for conducting and reporting systematic reviews.

Most of the included studies were from Asia, Europe and USA, so the conclusions may not be true for other ethnic groups. Besides, between-study heterogeneity could not be completely explained.

Introduction

The progression of CHD is complicated and is influenced by multi genetic and environmental factors, and atherosclerosis of coronary artery is the basic pathogenic factor of CHD¹. Plasma lipids and lipoproteins are important risk factors for atherosclerosis, and genes involved in lipoprotein metabolism might be candidate genes for CHD susceptibility^{2 3}. Apolipoprotein C3 (ApoC3) is an essential component of circulating particles in TG-rich lipoprotein, and inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by Apolipoprotein E⁴. Therefore, high levels of ApoC3 may cause hypertriglyceridemia⁵. Previous studies supported that ApoC3 might play an important role in CHD development, and the ApoC3 concentration was also associated with CHD risk^{6 7}.

Serum ApoC3 concentration was shown to be influenced by genetic and acquired factors⁸. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism⁹⁻¹². In recent years, studies have investigated the correlation between these polymorphisms and CHD risk, while the results were inconsistent. The aim of our meta-analysis was to assess the association between ApoC3 polymorphisms and risk of CHD.

Methods

Literature search

Systematic literature searches were conducted before March 2013 in Pubmed, Embase and Cochrane library databases without restrictions. Combination of the following terms were applied: “coronary heart disease” OR “coronary artery disease” OR “myocardial infarction” OR “acute coronary syndrome” OR “ischemic heart disease” OR “cardiovascular disease” OR “major adverse cardiac event” OR “CHD” OR “CAD” OR “MI” OR “ACS” OR “IHD” OR “MACE”; “apolipoprotein C3” OR “apolipoprotein C” OR “apolipoprotein C- III” OR “apolipoprotein C III” OR “APO C3” OR “APOC3” OR “APOC” OR “APO C III” OR “APO C- III”; “polymorphism” OR “variant” OR “SNP” OR “mutation”. References of relevant articles were also scanned for studies potentially missed in the primary searches. Articles published in English were retrieved. And the retrieved studies were carefully examined to exclude potential duplicates or overlapping data. This meta-analysis was designed, conducted and reported according to PRISMA statement¹³.

Selection criteria, data extraction and study quality assessment

Studies retrieved from the initial search were then screened for eligible articles. Titles and abstracts were scanned and then full papers were reviewed. We included articles if they met all the following criteria: (1) evaluating the association between ApoC3 polymorphism and coronary heart disease and (or) MI; (2) odds ratio (OR) estimates and their 95% confidence intervals (95%CI) were available or could be calculated; (3) each polymorphism included in the meta-analysis should be reported

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4 by at least two studies.

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6 Data were extracted independently by two reviewers (Bin Lin and Yiwei Huang).
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8 The following information was extracted from each study: first author, publication
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10 year, country, study design, sample size, gender distribution, mean age, phenotype
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12 (disease), genotype of cases and controls, whether the polymorphism(s) evaluated was
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14 in Hardy-Weinberg equilibrium or not, and the genotyping assay method. Any
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16 discrepancy was resolved by a third investigator. Newcastle-Ottawa Scale (NOS)
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18 method was applied to assess the study quality¹⁴. The NOS contains eight items and
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20 the score ranged from 0 to 9.
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26 **Statistical analysis**

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29 Dominant model was applied in this study as this genetic model was most widely
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31 used in the included studies. Because some studies did not apply dominant model, we
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33 re-calculated OR values under dominant model. Either a fixed-effects model or a
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35 random-effects model was applied to pool the OR estimates of each study, according
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37 to heterogeneity across studies. The extent of heterogeneity was checked using the
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39 chi-square test and I^2 test; $p \leq 0.10$ and/or $I^2 > 50\%$ indicates significant heterogeneity.
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42 When $p > 0.10$, the fixed-effects model was applied and otherwise we used the
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44 random-effects model. Subgroup analysis was applied to explore heterogeneity.
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47 Study-specific ORs of CHD were firstly pooled and then we evaluated the association
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49 between ApoC3 polymorphisms and MI risk separately. Funnel plots were constructed
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51 and Begg's and Egger's tests were used to assess publication bias, and $p \leq 0.10$ was
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53 considered to be significant. All analyses were conducted using the Stata software
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(version 11.0; StatCorp, College Station, TX, USA).

Results

Study selection and characteristics

A total of 1532 articles were identified by searching Pubmed, Embase and Cochrane library databases. Among them, 1482 articles were excluded through screening titles and abstracts. The remaining 50 articles were carefully evaluated as full texts and 30 articles were excluded. The reasons for exclusion were articles not on right topic (15 papers), insufficient data (6 papers), relevant reviews (8 papers) and duplicate reports from the same study (1 paper). This meta-analysis finally included 20 articles^{9-12 15-30}. The selection process was shown in Figure 1 while the characteristics of those studies were listed in Supplementary Table 1. Among these studies, fifteen studies assessed ApoC3 Sst I polymorphism, four studies evaluated ApoC3 T-455C polymorphism, four studies reported ApoC3 C-482T polymorphism and three investigated ApoC3 C1100T polymorphism (several studies reported more than one polymorphism). The results of quality assessment were shown in the Supplementary Table 2 and the score of the included studies ranged from 5 to 9.

AOPC3 polymorphisms and risk of CHD

Meta-analysis of Sst I polymorphism

A total of 15 studies with 11539 individuals assessed the association between Sst I polymorphism and CHD risk. Fourteen studies were in Hardy-Weinberg equilibrium while one study did not report whether Sst I polymorphism was in Hardy-Weinberg equilibrium or not²². Most of the study used restriction fragment length polymorphism

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4 (RFLP) method for DNA genotyping (n=14) and one study applied immobilized
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6 oligonucleotide probes array (IOPA) method²⁴. Multivariable OR could be extracted
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8 from 4 studies^{10 20 22 24} (Supplementary Table 3). Under dominant model (S1S2+S2S2
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10 vs. S1S1), the pooled univariate OR of all studies was 1.19 (95% CI 1.00-1.42)
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12 (Figure 2, Table 1), indicating a borderline significant association between Sst I
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14 polymorphism and CHD risk. There was significant heterogeneity among studies ($I^2 =$
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16 48.9%, $p=0.017$) (Figure 2, Table 1). The pooled multivariable OR was 1.11
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18 (0.73-1.70), did not suggest a significant association.
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24 According to study characteristics, subgroup analysis was adopted, as shown in
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26 Table 2. Pooled results showed that S1S2 and S2S2 genotypes might increase risk of
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28 CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian
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30 population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence
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32 the result, Sst I polymorphism was significantly associated with CHD risk in
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34 retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective
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36 studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was
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38 observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI
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40 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35). After excluding
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42 the study did not report Hardy-Weinberg equilibrium, the pooled OR was 1.24 (95%
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44 CI 1.04-1.47). The pooled OR of the studies applied RFLP method was 1.19 (95% CI
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46 0.98-1.44).
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53 54 **Meta-analysis of T-455C polymorphism**

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56 The association between T-455C polymorphism and CHD risk was evaluated by 4
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4 studies comprising 3378 individuals. All the studies were in Hardy-Weinberg
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6 equilibrium. Immobilized oligonucleotide probes array method was used by three
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8 studies while real-time fluorescence quantitative PCR was applied by one study⁹.
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10 Only one study reported a multivariable OR of 1.82 (95% CI 1.05-3.18), while the
11
12 unadjusted OR was 1.15 (98% CI 0.85-1.55)¹⁷ (Supplementary Table 3). Results
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14 indicated significant association between T-455C polymorphism and CHD risk
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16 (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42) (Figure 3, Table 1). No
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18 significant heterogeneity among studies was indicated ($I^2 = 0\%$, $p=0.580$) (Figure 3,
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20 Table 1). Two studies reported the association between T-455C polymorphism and
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22 MI risk, and the pooled result suggested a borderline significant association (pooled
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24 OR=1.21, 95% CI 1.00-1.46).
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31 **Meta-analysis of C-482T polymorphism**

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34 Four studies with 3070 individuals reported the association between C-482T
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36 polymorphism and CHD risk. Only one study did not report whether C-482T
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38 polymorphism was in Hardy-Weinberg equilibrium or not²². Two studies applied
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40 real-time fluorescence quantitative PCR method^{9 16}, one study used RFLP method²²
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42 and the other study adopted immobilized oligonucleotide probes array method¹⁹.
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44 Multivariable OR could be extracted from two studies^{16 18} (Supplementary Table 3).
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46 There was no significant association between C-482T polymorphism and CHD risk
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48 (CT+TT vs. CC, pooled OR=1.06, 95% CI 0.92-1.22) (Figure 4, Table 1). No
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50 significant heterogeneity was observed ($I^2 = 0\%$, $p=0.788$) (Figure 4, Table 1). Only
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52 one study reported the association between C-482T polymorphism and MI risk
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(OR=1.12, 95% CI 0.88-1.43)¹⁹.

Meta-analysis of C1100T polymorphism

Three studies comprising 4662 participants evaluated the association between C1100T polymorphism and CHD risk. Two studies adopted immobilized oligonucleotide probes array method^{19,24} and one study used RFLP method²². No significant association was found (CT+TT vs. CC, pooled OR=1.06, 95% CI 0.89-1.27) and no significant heterogeneity was observed ($I^2 = 46.7\%$, $p=0.153$) (Figure 5, Table 1). One study evaluated the association between C1100T polymorphism and MI risk (OR=1.18, 95% CI 0.93-1.51)¹⁹.

Publication bias

Begg's and Egger's tests suggested that no publication bias was found in our meta-analyses.

Discussion

ApoC3 is a glycoprotein synthesized mainly in the liver and the intestinal, and plays an essential role in regulating the serum triglyceride levels. Besides, it can strongly regulate the levels of VLDL and small dense LDL which potentially improves atherosclerosis³¹. Clinical research found that ApoC3 levels were predictor of risk for development of CHD³²⁻³⁴. In the present study, twenty studies were included and four polymorphisms of ApoC3 were evaluated, including C-482T, T-455C, Sst I and C1100T polymorphisms. T-455C polymorphism was suggested to be significantly associated with CHD risk, and 'C' allele increased CHD risk by 22 percent (CT+TT vs. CC, pooled OR=1.22, 95% CI 1.06-1.42). A borderline

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4 significant association was observed between Sst I polymorphism and CHD risk.
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6 While no evidence suggested significant association between C-482T and C1100T
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8 polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism,
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10 and we found that Sst I polymorphism was significantly associated with MI risk.
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12 Besides, Sst I polymorphism was significantly associated with CHD risk in Asian
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14 population but not in Caucasian population, indicating that the effect of Sst I
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16 polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was
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18 indicated to be significantly associated with CHD risk but this association was not
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20 confirmed in prospective studies. So, the association between Sst I polymorphism and
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22 CHD risk should be interpreted cautiously.
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29 The mechanism of Sst I polymorphism in CHD susceptibility may be multiple.
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31 Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is
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33 possible that this polymorphism is in linkage disequilibrium with other functional
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35 polymorphism in the nearby region, such as T-455C polymorphism¹⁰. Sst I
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37 polymorphism might alter plasma lipid concentrations. Several studies showed S2
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39 carriers have higher plasma total cholesterol, TG and LDL-C levels^{12 35 36}, though
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41 other studies did not demonstrate significant difference^{10 20 37}. Besides, it has been
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43 shown that S2 allele might significantly influence dyslipidemic state and
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45 atherosclerosis severity when patients changed their diet from saturated fatty acids to
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47 olive oil³⁸. So, Sst I polymorphism plays an important role in modulating lipid levels
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49 response to dietary changes.
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56 T-455C and C-482T polymorphisms both located in the 5' promoter region and
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4 were in strong linkage disequilibrium with each other. These two polymorphisms
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6 have been studied extensively because they could alter the nuclear transcript factors
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8 which mediate the insulin response. Significant association between T-455C
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10 polymorphism and risk of CHD was found under dominant model. However, it should
11
12 be noted that in the 4 studies evaluating T-455C polymorphism, only one study²⁴
13
14 showed a significant association between T-455C polymorphism and CHD risk. So,
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16 more studies with large sample size are warranted to clarify this issue. For C-482T
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18 polymorphism, no significant association with CHD risk was found.
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24 Different mechanisms could be linked to this finding. In previous works, T-455C
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26 polymorphism was associated with increased TG and ApoC3 levels^{24 39}. Also, T-455C
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28 polymorphism was demonstrated to be significantly associated with metabolic
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30 syndrome³⁹. Another study showed T-455C polymorphism could interfere with n-3
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32 polyunsaturated fatty acids on ApoC3 concentrations⁷. Olivieri O *et al.* found that CC
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34 homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3
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36 polyunsaturated fatty acids⁷.
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42 The present meta-analysis has several strengths. First, this study was based on
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44 guidelines for conducting and reporting systematic reviews and the methods were
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46 rigorous. Second, we comprehensively evaluated the association between ApoC3
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48 polymorphisms and CHD risk, and a total of four polymorphisms of ApoC3 were
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50 assessed. Besides, no publication bias was observed, indicating the pooled results
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52 might be unbiased.
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57 The current analysis also has several limitations. First, most of the included studies
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3 investigated Asian or Caucasian population, so the conclusions may not be true for
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5 other ethnic groups. Second, significant heterogeneity was found and could not be
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7 completely explained when assess some polymorphisms. Third, only Pubmed,
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9 Embase and Cochrane library were searched for eligible articles. Finally, only articles
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11 published in English were included.
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16 Some questions remain unanswered in the present study. Most of the studies did not
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18 report multivariable OR, so it is not clear whether ApoC3 polymorphisms could be an
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20 independent predictor of CHD risk or not. More studies are warranted to clarify this
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22 issue. Different genotyping assay methods were applied in the included studies, which
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24 might call for different results. And it should be noted that ApoC3 polymorphisms are
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26 non-modified risk factors, and little control methods over them were accessible.
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28 However, people in high risk groups (such as S2 carriers of Sst I polymorphism)
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30 might be advised to go for regular checkups to reduce the risk of adverse cardiac
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32 event. Detecting ApoC3 polymorphisms may help people be aware of the risk of CHD.
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34 With the current level of evidence, we cannot comment on the optimal genotyping
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36 assay method and the cost-effectiveness of detecting ApoC3 polymorphisms. Further
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38 research is needed to explore combination of variables associated with CHD risk to
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40 develop a predictive model with a high discriminative capacity.
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49 In summary, ApoC3 Sst I and T-455C polymorphisms might be associated with
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51 CHD risk, while no evidence suggested significant association between C-482T and
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53 C1100T polymorphisms and CHD risk.
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Table 1 Meta-analysis results of Sst I, T-455C, C-482T, C1100T polymorphisms

Polymorphism	No. of studies	No. of participants	Comparison	OR (95% CI)	Heterogeneity	
					I ² (%)	P
Sst I	15	11539	S1S2+S2S2 vs. S1S1	1.19 (1.00-1.42)	48.9	0.017
T-455C	4	3378	TC+CC vs. TT	1.22 (1.06-1.42)	0	0.580
C-482T	4	3070	CT+TT vs. CC	1.06 (0.92-1.22)	0	0.788
C1100T	3	4662	CT+TT vs. CC	1.06 (0.89-1.27)	46.7	0.153

Table 2 Subgroup analysis of Sst I polymorphism

Groups		OR (95% CI)	Heterogeneity	
			I ² (%)	P
Ethnicity	Caucasian	1.14 (0.92-1.41)	54.2	0.013
	Asian	1.35 (1.08-1.69)	0	0.427
Study design	Retrospective	1.30 (1.04-1.61)	39.0	0.098
	Prospective	0.98 (0.75-1.28)	52.3	0.098
Phenotype	CHD	1.09 (0.87-1.35)	46.0	0.047
	MI	1.42 (1.06-1.91)	52.3	0.099

Figure legends

Figure 1. Flow diagram of study selection process.

Figure 2. Association between Sst I polymorphism and CHD risk.

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7 **Figure 3.** Association between T-455C polymorphism and CHD risk.
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10 **Figure 4.** Association between C-482T polymorphism and CHD risk.
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12 **Figure 5.** Association between C1100T polymorphism and CHD risk.
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17 **Supplementary file.**
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20 **Supplementary Table 1** Characteristics of the included studies
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22 **Supplementary Table 2** Quality assessment of the included studies
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24 **Supplementary Table 3** Studies reporting multivariable OR
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27 **Checklist** PRISMA checklist
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4 **Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T**

5
6 **polymorphisms and risk of coronary heart disease**

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8
9 Bin Lin^{1*}, Yiwei Huang¹, Mingying Zhang¹, Jun Wang¹, Yihua Wu^{2*}

10
11 ¹Department of Cardiology, Wenzhou Central Hospital, Wenzhou, 325000 China;

12
13 ²Department of Epidemiology and Health Statistics, Zhejiang University School of
14
15 Public Health, Hangzhou, 310058 China;

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17
18 (Running title: ApoC3 polymorphisms and CHD risk)

19
20
21 *Corresponding Authors:

22
23
24 Bin Lin, MD, Department of Cardiology, Wenzhou Central Hospital, Wenzhou,
25
26 325000 China;

27
28
29 Tel: 13706670776

30
31 E-mail: d4c3b2a@sina.com

32
33
34 Yihua Wu, PhD, Department of Epidemiology and Health Statistics, Zhejiang
35
36 University School of Public Health, Hangzhou, 310058 China;

37
38
39 Tel/Fax: 86-0571-88208140

40
41
42 E-mail: georgewuer@126.com

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46 We declare that we have no conflict of interest.
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Abstract

Objectives: Apolipoprotein C3 (ApoC3) polymorphisms have been suggested to be associated with risk of coronary heart disease (CHD). However, the results of relevant studies were inconsistent. We aimed to systematically evaluate this issue.

Design: Pubmed, Embase and Cochrane library databases (up to March 2013) were systematically searched to identify studies evaluating the association between ApoC3 polymorphisms and CHD risk. Two reviewers independently identified studies, extracted and analyzed data. Either a fixed- or a random-effects model was adopted to estimate overall odds ratios (ORs).

Studies reviewed: Finally, twenty studies comprising 15591 participants were included in this systematic review. Fifteen studies with 11539 individuals were included in the meta-analysis of Sst I polymorphism, four studies comprising 3378 individuals assessed T-455C polymorphism, four studies with 3070 participants evaluated C-482T polymorphism and C1100T polymorphism was assessed by 3 studies comprising 4662 participants.

Results: Under dominant model, Sst I polymorphism was borderline significantly associated with CHD risk (S1S2+S2S2 vs. S1S1, pooled OR=1.19, 95% CI 1.00-1.42). Subgroup analyses suggested that Sst I polymorphism was significantly associated with myocardial infarction (MI) risk (pooled OR=1.42, 95% CI 1.06-1.91), and Sst I polymorphism was statistically associated with CHD risk among Asian population (pooled OR=1.35, 95% CI 1.08-1.69) and in retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61). Significant association was observed between T-455C

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4 polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42).

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6 A borderline significant association was suggested between T-455C polymorphism
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8 and MI risk (pooled OR=1.21, 95% CI 1.00-1.46). C-482T and C1100T
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10 polymorphisms were not indicated to be associated with CHD risk or MI risk.
11

12
13 **Conclusions:** ApoC3 Sst I and T-455C polymorphisms might be associated with
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15 CHD risk.
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18 **Key Words:** coronary heart disease, apolipoprotein C-III, genetic polymorphisms,
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20 risk factors, meta-analysis.
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23 24 25 **Article focus**

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27 The aim of this study was to systematically evaluate the association between
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29 apolipoprotein C3 polymorphisms and CHD risk.
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32 33 **Key message**

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35 This study shows that ApoC3 Sst I and T-455C polymorphisms might be associated
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37 with CHD risk.
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40 41 **Strengths and limitations of the study**

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43 The present study comprehensively evaluated the association between ApoC3
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45 polymorphisms, including Sst I, T-455C, C-482T, C1100T and CHD risk. The
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47 methods of this study were rigorous and were based on guidelines for conducting and
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49 reporting systematic reviews.
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53 Most of the included studies were from Asia, Europe and USA, so the conclusions
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55 may not be true for other ethnic groups. Besides, between-study heterogeneity could
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57 not be completely explained.
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Introduction

The progression of CHD is complicated and is influenced by multi genetic and environmental factors, and atherosclerosis of coronary artery is the basic pathogenic factor of CHD¹. Plasma lipids and lipoproteins are important risk factors for atherosclerosis, and genes involved in lipoprotein metabolism might be candidate genes for CHD susceptibility^{2 3}. Apolipoprotein C3 (ApoC3) is an essential component of circulating particles in TG-rich lipoprotein, and inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by Apolipoprotein E⁴. Therefore, high levels of ApoC3 may cause hypertriglyceridemia⁵. Previous studies supported that ApoC3 might play an important role in CHD development, and the ApoC3 concentration was also associated with CHD risk^{6 7}.

Serum ApoC3 concentration was shown to be influenced by genetic and acquired factors⁸. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism⁹⁻¹². In recent years, studies have investigated the **correlation** between these polymorphisms and CHD risk, while the results were inconsistent. The aim of our meta-analysis was to assess the association between ApoC3 polymorphisms and risk of CHD.

Methods

Literature search

Systematic literature searches were conducted before March 2013 in Pubmed, Embase **and Cochrane library** databases without restrictions. Combination of the following terms were applied: “coronary heart disease” OR “coronary artery disease”

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4 OR “myocardial infarction” OR “acute coronary syndrome” OR “ischemic heart
5
6 disease” OR “cardiovascular disease” OR “major adverse cardiac event” OR “CHD”
7
8 OR “CAD” OR “MI” OR “ACS” OR “IHD” OR “MACE”; “apolipoprotein C3” OR
9
10 “apolipoprotein C” OR “apolipoprotein C- III” OR “apolipoprotein C III” OR “APO
11
12 C3” OR “APOC3” OR “APOC” OR “APO C III” OR “APO C- III”; “polymorphism”
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14 OR “variant” OR “SNP” OR “mutation”. References of relevant articles were also
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16 scanned for studies potentially missed in the primary searches. Articles published in
17
18 English were retrieved. And the retrieved studies were carefully examined to exclude
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20 potential duplicates or overlapping data. This meta-analysis was designed, conducted
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22 and reported according to PRISMA statement¹³.
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29 **Selection criteria, data extraction and study quality assessment**

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31 Studies retrieved from the initial search were then screened for eligible articles.
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33 Titles and abstracts were scanned and then full papers were reviewed. We included
34
35 articles if they met all the following criteria: (1) evaluating the association between
36
37 ApoC3 polymorphism and coronary heart disease and (or) MI; (2) odds ratio (OR)
38
39 estimates and their 95% confidence intervals (95%CI) were available or could be
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41 calculated; (3) each polymorphism included in the meta-analysis should be reported
42
43 by at least two studies.
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49 Data were extracted independently by two reviewers (Bin Lin and Yiwei Huang).
50
51 The following information was extracted from each study: first author, publication
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53 year, country, study design, sample size, gender distribution, mean age, phenotype
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55 (disease), genotype of cases and controls, whether the polymorphism(s) evaluated was
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4 in Hardy-Weinberg equilibrium or not, and the genotyping assay method. Any
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6 discrepancy was resolved by a third investigator. Newcastle-Ottawa Scale (NOS)
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8 method was applied to assess the study quality¹⁴. The NOS contains eight items and
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10 the score ranged from 0 to 9.
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12 13 14 **Statistical analysis**

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16 Dominant model was applied in this study as this genetic model was most widely
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18 used in the included studies. Because some studies did not apply dominant model, we
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20 re-calculated OR values under dominant model. Either a fixed-effects model or a
21
22 random-effects model was applied to pool the OR estimates of each study, according
23
24 to heterogeneity across studies. The extent of heterogeneity was checked using the
25
26 chi-square test and I^2 test; $p \leq 0.10$ and/or $I^2 > 50\%$ indicates significant heterogeneity.
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28 When $p > 0.10$, the fixed-effects model was applied and otherwise we used the
29
30 random-effects model. Subgroup analysis was applied to explore heterogeneity.
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36 Study-specific ORs of CHD were firstly pooled and then we evaluated the association
37
38 between ApoC3 polymorphisms and MI risk separately. Funnel plots were constructed
39
40 and Begg's and Egger's tests were used to assess publication bias, and $p \leq 0.10$ was
41
42 considered to be significant. All analyses were conducted using the Stata software
43
44 (version 11.0; StatCorp, College Station, TX, USA).
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48 49 **Results**

50 51 **Study selection and characteristics**

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54 A total of 1532 articles were identified by searching Pubmed, Embase and
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56 Cochrane library databases. Among them, 1482 articles were excluded through
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4 screening titles and abstracts. The remaining 50 articles were carefully evaluated as
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6 full texts and 30 articles were excluded. The reasons for exclusion were articles not on
7
8 right topic (15 papers), insufficient data (6 papers), relevant reviews (8 papers) and
9
10 duplicate reports from the same study (1 paper). This meta-analysis finally included
11
12 20 articles^{9-12 15-30}. The selection process was shown in Figure 1 while the
13
14 characteristics of those studies were listed in Supplementary Table 1. Among these
15
16 studies, fifteen studies assessed ApoC3 Sst I polymorphism, four studies evaluated
17
18 ApoC3 T-455C polymorphism, four studies reported ApoC3 C-482T polymorphism
19
20 and three investigated ApoC3 C1100T polymorphism (several studies reported more
21
22 than one polymorphism). The results of quality assessment were shown in the
23
24 Supplementary Table 2 and the score of the included studies ranged from 5 to 9.
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31 **AOPC3 polymorphisms and risk of CHD**

32 **Meta-analysis of Sst I polymorphism**

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36 A total of 15 studies with 11539 individuals assessed the association between Sst I
37
38 polymorphism and CHD risk. Fourteen studies were in Hardy-Weinberg equilibrium
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40 while one study did not report whether Sst I polymorphism was in Hardy-Weinberg
41
42 equilibrium or not²². Most of the study used restriction fragment length polymorphism
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44 (RFLP) method for DNA genotyping (n=14) and one study applied immobilized
45
46 oligonucleotide probes array (IOPA) method²⁴. Multivariable OR could be extracted
47
48 from 4 studies^{10 20 22 24} (Supplementary Table 3). Under dominant model (S1S2+S2S2
49
50 vs. S1S1), the pooled univariate OR of all studies was 1.19 (95% CI 1.00-1.42)
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52 (Figure 2, Table 1), indicating a borderline significant association between Sst I
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3 polymorphism and CHD risk. There was significant heterogeneity among studies ($I^2 =$
4 48.9%, $p=0.017$) (Figure 2, Table 1). **The pooled multivariable OR was 1.11**
5
6 **(0.73-1.70), did not suggest a significant association.**
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11 According to study characteristics, subgroup analysis was **adopted**, as shown in
12
13 Table 2. Pooled **results** showed that S1S2 and S2S2 genotypes might increase risk of
14
15 CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian
16
17 population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence
18
19 the result, Sst I polymorphism was significantly associated with CHD risk in
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21 retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective
22
23 studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was
24
25 observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI
26
27 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35). **After excluding**
28
29 **the study did not report Hardy-Weinberg equilibrium, the pooled OR was 1.24 (95%**
30
31 **CI 1.04-1.47). The pooled OR of the studies applied RFLP method was 1.19 (95% CI**
32
33 **0.98-1.44).**
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41 **Meta-analysis of T-455C polymorphism**

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43 The association between T-455C polymorphism and CHD risk was evaluated by 4
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45 studies comprising 3378 individuals. **All the studies were in Hardy-Weinberg**
46
47 **equilibrium. Immobilized oligonucleotide probes array method was used by three**
48
49 **studies while real-time fluorescence quantitative PCR was applied by one study⁹.**
50
51 **Only one study reported a multivariable OR of 1.82 (95% CI 1.05-3.18), while the**
52
53 **unadjusted OR was 1.15 (98% CI 0.85-1.55)¹⁷ (Supplementary Table 3). Results**
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3 indicated significant association between T-455C polymorphism and CHD risk
4 (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42) (Figure 3, Table 1). No
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6 (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42) (Figure 3, Table 1). No
7
8 significant heterogeneity among studies was indicated ($I^2 = 0\%$, $p=0.580$) (Figure 3,
9
10
11 Table 1). Two studies reported the association between T-455C polymorphism and
12
13 MI risk, and the pooled result suggested a borderline significant association (pooled
14
15 OR=1.21, 95% CI 1.00-1.46).
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18 19 **Meta-analysis of C-482T polymorphism**

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21 Four studies with 3070 individuals reported the association between C-482T
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23 polymorphism and CHD risk. Only one study did not report whether C-482T
24
25 polymorphism was in Hardy-Weinberg equilibrium or not²². Two studies applied
26
27 real-time fluorescence quantitative PCR method^{9 16}, one study used RFLP method²²
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29 and the other study adopted immobilized oligonucleotide probes array method¹⁹.
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31
32 Multivariable OR could be extracted from two studies^{16 18} (Supplementary Table 3).
33
34 There was no significant association between C-482T polymorphism and CHD risk
35
36 (CT+TT vs. CC, pooled OR=1.06, 95% CI 0.92-1.22) (Figure 4, Table 1). No
37
38 significant heterogeneity was observed ($I^2 = 0\%$, $p=0.788$) (Figure 4, Table 1). Only
39
40 one study reported the association between C-482T polymorphism and MI risk
41
42 (OR=1.12, 95% CI 0.88-1.43)¹⁹.
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48 49 **Meta-analysis of C1100T polymorphism**

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51 Three studies comprising 4662 participants evaluated the association between
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53 C1100T polymorphism and CHD risk. Two studies adopted immobilized
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55 oligonucleotide probes array method^{19 24} and one study used RFLP method²². No
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4 significant association was found (CT+TT vs. CC, pooled OR=1.06, 95% CI
5
6 0.89-1.27) and no significant heterogeneity was observed ($I^2 = 46.7\%$, $p=0.153$)
7
8 (Figure 5, Table 1). One study evaluated the association between C1100T
9
10 polymorphism and MI risk (OR=1.18, 95% CI 0.93-1.51)¹⁹.
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12

13 14 **Publication bias**

15
16 Begg's and Egger's tests suggested that no publication bias was found in our
17
18 meta-analyses.
19

20 21 **Discussion**

22
23 ApoC3 is a glycoprotein synthesized mainly in the liver and the intestinal, and
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25 plays an essential role in regulating the serum triglyceride levels. Besides, it can
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27 strongly regulate the levels of VLDL and small dense LDL which potentially
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29 improves atherosclerosis³¹. Clinical research found that ApoC3 levels were predictor
30
31 of risk for development of CHD³²⁻³⁴. In the present study, twenty studies were
32
33 included and four polymorphisms of ApoC3 were evaluated, including C-482T,
34
35 T-455C, Sst I and C1100T polymorphisms. T-455C polymorphism was suggested to
36
37 be significantly associated with CHD risk, and 'C' allele increased CHD risk by 22
38
39 percent (CT+TT vs. CC, pooled OR=1.22, 95% CI 1.06-1.42). A borderline
40
41 significant association was observed between Sst I polymorphism and CHD risk.
42
43 While no evidence suggested significant association between C-482T and C1100T
44
45 polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism,
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47 and we found that Sst I polymorphism was significantly associated with MI risk.
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49 Besides, Sst I polymorphism was significantly associated with CHD risk in Asian
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4 population but not in Caucasian population, indicating that the effect of Sst I
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6 polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was
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8 indicated to be significantly associated with CHD risk but this association was not
9
10 confirmed in prospective studies. So, the association between Sst I polymorphism and
11
12 CHD risk should be interpreted cautiously.
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16 The mechanism of Sst I polymorphism in CHD susceptibility may be multiple.
17
18 Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is
19
20 possible that this polymorphism is in linkage disequilibrium with other functional
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22 polymorphism in the nearby region, such as T-455C polymorphism¹⁰. Sst I
23
24 polymorphism might alter plasma lipid concentrations. Several studies showed S2
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26 carriers have higher plasma total cholesterol, TG and LDL-C levels^{12 35 36}, though
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28 other studies did not demonstrate significant difference^{10 20 37}. Besides, it has been
29
30 shown that S2 allele might significantly influence dyslipidemic state and
31
32 atherosclerosis severity when patients changed their diet from saturated fatty acids to
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34 olive oil³⁸. So, Sst I polymorphism plays an important role in modulating lipid levels
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36 response to dietary changes.
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44 T-455C and C-482T polymorphisms both located in the 5' promoter region and
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46 were in strong linkage disequilibrium with each other. These two polymorphisms
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48 have been studied extensively because they could alter the nuclear transcript factors
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50 which mediate the insulin response. Significant association between T-455C
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52 polymorphism and risk of CHD was found under dominant model. However, it should
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54 be noted that in the 4 studies evaluating T-455C polymorphism, only one study²⁴
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3 showed a significant association between T-455C polymorphism and CHD risk. So,
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5 more studies with large sample size are warranted to clarify this issue. For C-482T
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8 polymorphism, no significant association with CHD risk was found.
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11 Different mechanisms could be linked to this finding. In previous works, T-455C
12
13 polymorphism was associated with increased TG and ApoC3 levels^{24,39}. Also, T-455C
14
15 polymorphism was demonstrated to be significantly associated with metabolic
16
17 syndrome³⁹. Another study showed T-455C polymorphism could interfere with n-3
18
19 polyunsaturated fatty acids on ApoC3 concentrations⁷. Olivieri O *et al.* found that CC
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21 homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3
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23 polyunsaturated fatty acids⁷.
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29 The present meta-analysis has several strengths. First, this study was based on
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31 guidelines for conducting and reporting systematic reviews and the methods were
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33 rigorous. Second, we comprehensively evaluated the association between ApoC3
34
35 polymorphisms and CHD risk, and a total of four polymorphisms of ApoC3 were
36
37 assessed. Besides, no publication bias was observed, indicating the pooled results
38
39 might be unbiased.
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44 The current analysis also has several limitations. First, most of the included studies
45
46 investigated Asian or Caucasian population, so the conclusions may not be true for
47
48 other ethnic groups. Second, significant heterogeneity was found and could not be
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50 completely explained when assess some polymorphisms. Third, only Pubmed,
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52 Embase and Cochrane library were searched for eligible articles. Finally, only articles
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54 published in English were included.
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Some questions remain unanswered in the present study. Most of the studies did not report multivariable OR, so it is not clear whether ApoC3 polymorphisms could be an independent predictor of CHD risk or not. More studies are warranted to clarify this issue. Different genotyping assay methods were applied in the included studies, which might call for different results. And it should be noted that ApoC3 polymorphisms are non-modified risk factors, and little control methods over them were accessible. However, people in high risk groups (such as S2 carriers of Sst I polymorphism) might be advised to go for regular checkups to reduce the risk of adverse cardiac event. Detecting ApoC3 polymorphisms may help people be aware of the risk of CHD. With the current level of evidence, we cannot comment on the optimal genotyping assay method and the cost-effectiveness of detecting ApoC3 polymorphisms. Further research is needed to explore combination of variables associated with CHD risk to develop a predictive model with a high discriminative capacity.

In summary, ApoC3 Sst I and T-455C polymorphisms might be associated with CHD risk, while no evidence suggested significant association between C-482T and C1100T polymorphisms and CHD risk.

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4 the manuscript.

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6 **Data sharing statement:** No additional data are available.
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9 We declare that we have no conflict of interest.
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Table 1 Meta-analysis results of Sst I, T-455C, C-482T, C1100T polymorphisms

Polymorphism	No. of studies	No. of participants	Comparison	OR (95% CI)	Heterogeneity	
					I ² (%)	P
Sst I	15	11539	S1S2+S2S2 vs. S1S1	1.19 (1.00-1.42)	48.9	0.017
T-455C	4	3378	TC+CC vs. TT	1.22 (1.06-1.42)	0	0.580
C-482T	4	3070	CT+TT vs. CC	1.06 (0.92-1.22)	0	0.788
C1100T	3	4662	CT+TT vs. CC	1.06 (0.89-1.27)	46.7	0.153

Table 2 Subgroup analysis of Sst I polymorphism

Groups		OR (95% CI)	Heterogeneity	
			I ² (%)	P
Ethnicity	Caucasian	1.14 (0.92-1.41)	54.2	0.013
	Asian	1.35 (1.08-1.69)	0	0.427
Study design	Retrospective	1.30 (1.04-1.61)	39.0	0.098
	Prospective	0.98 (0.75-1.28)	52.3	0.098
Phenotype	CHD	1.09 (0.87-1.35)	46.0	0.047
	MI	1.42 (1.06-1.91)	52.3	0.099

Figure legends

Figure 1. Flow diagram of study selection process.

Figure 2. Association between Sst I polymorphism and CHD risk.

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7 **Figure 3.** Association between T-455C polymorphism and CHD risk.
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9 **Figure 4.** Association between C-482T polymorphism and CHD risk.
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11 **Figure 5.** Association between C1100T polymorphism and CHD risk.
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17 **Supplementary file.**
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19 **Supplementary Table 1** Characteristics of the included studies
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21 **Supplementary Table 2** Quality assessment of the included studies
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23 **Supplementary Table 3** Studies reporting multivariable OR
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26 **Checklist** PRISMA checklist
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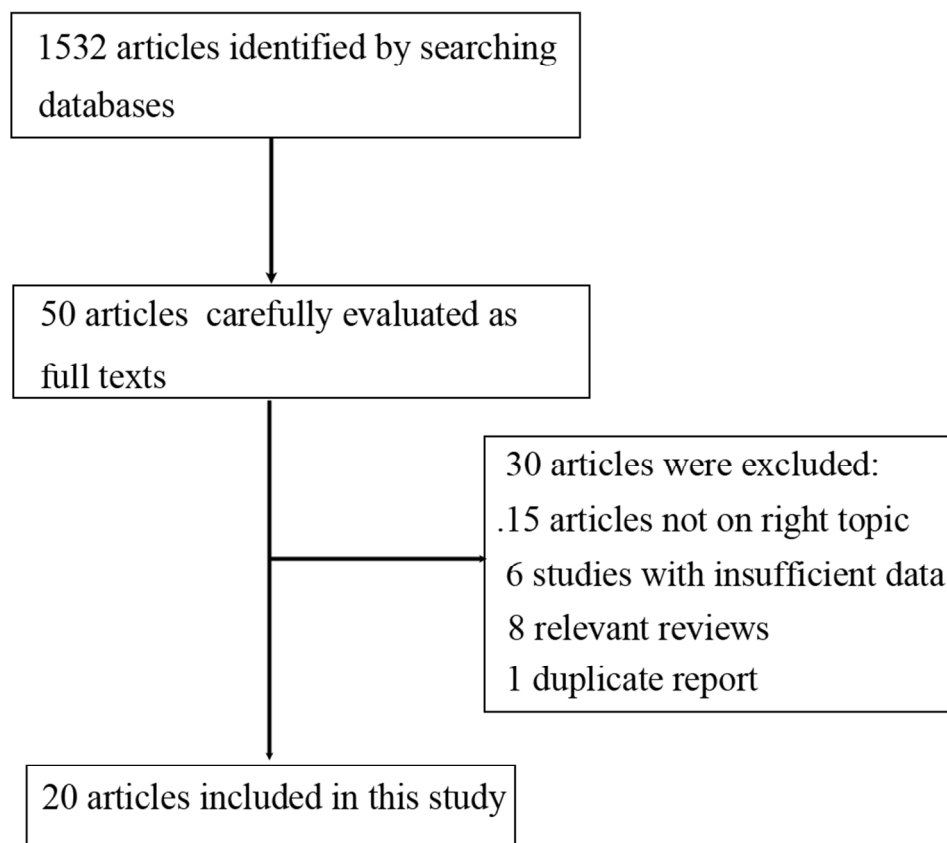


Figure 1. Flow diagram of study selection process.
88x88mm (300 x 300 DPI)

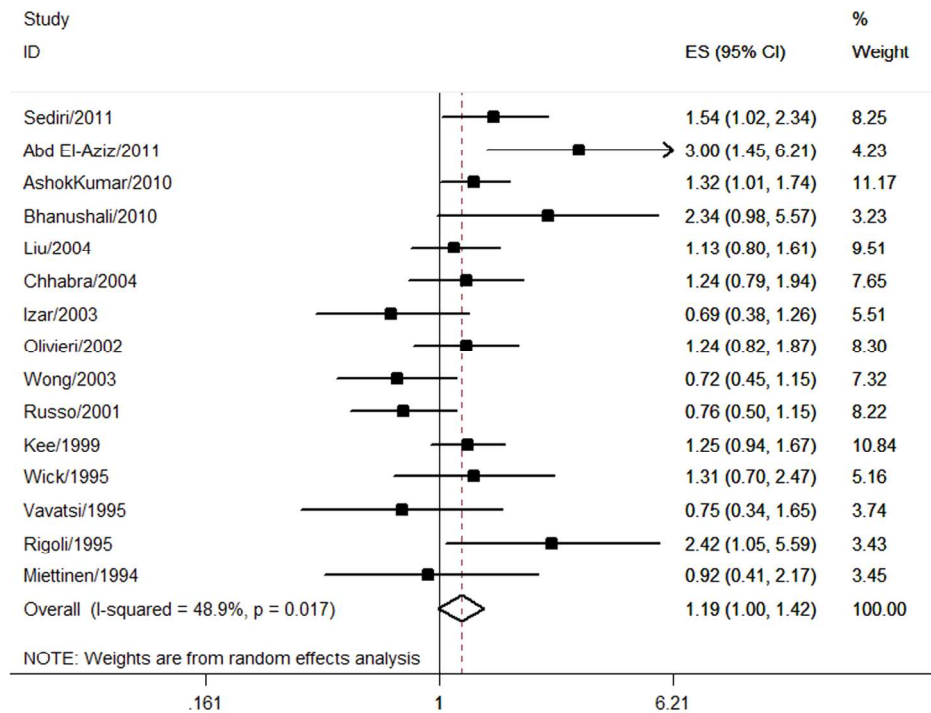


Figure 2. Association between Sst I polymorphism and CHD risk.
127x102mm (300 x 300 DPI)

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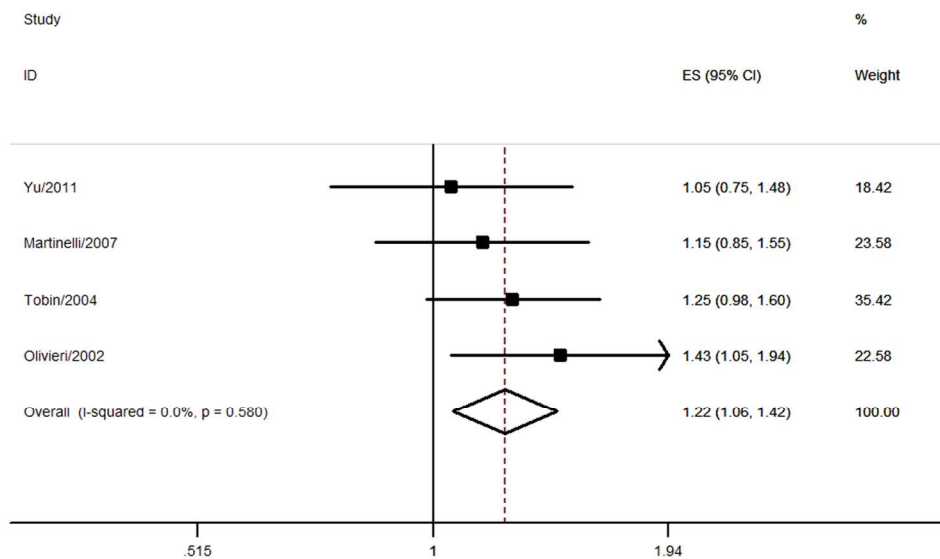


Figure 3. Association between T-455C polymorphism and CHD risk.
127x84mm (300 x 300 DPI)

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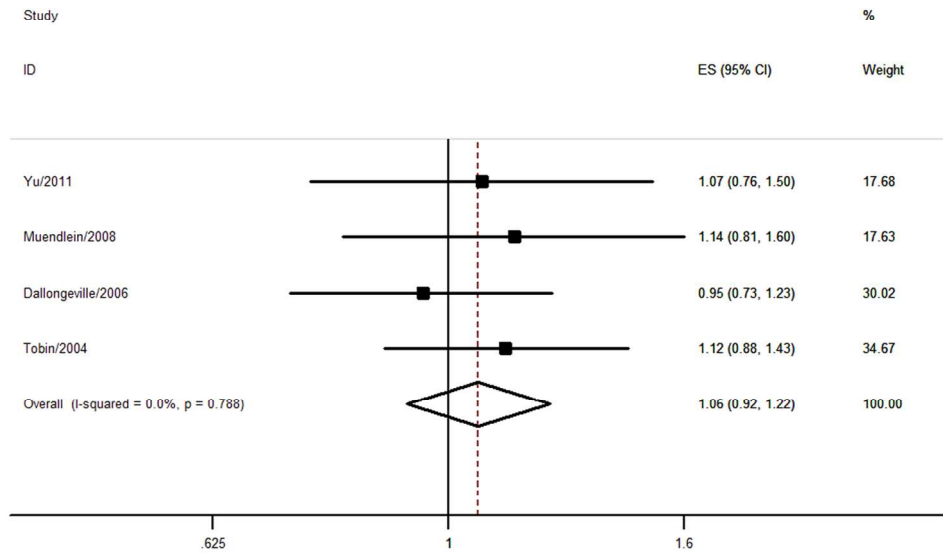


Figure 4. Association between C-482T polymorphism and CHD risk.
127x84mm (300 x 300 DPI)

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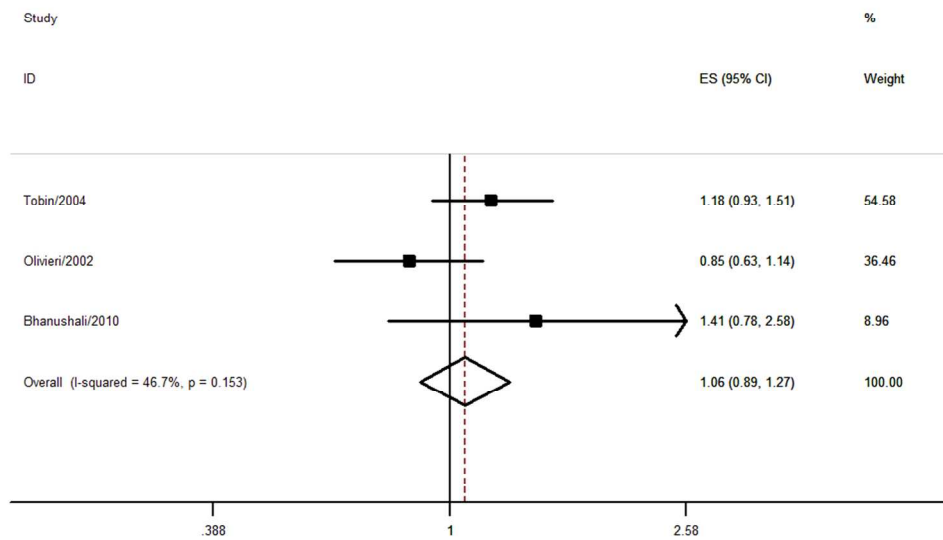


Figure 5. Association between C1100T polymorphism and CHD risk.
127x82mm (300 x 300 DPI)

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Supplementary Table 1 Characteristics of the included studies

Study and Publication year	Country	Study design	Sample size (cases/controls)	Sex (M/F)	Mean Age (years)	Phenotype	Mutation or polymorphisms	Cases			Controls			HWE	Method
								aa	ab*	bb	aa	ab	bb		
Yu/2011 ⁹	China	Case-control	611(286/325)	Cases: 214/72 Controls: 172/153	Cases: 56.3 Controls: 55.79	CHD	T-455C C-482T	90 89	13 13	47 48	11 11	157 159	54 52	Yes	Real-time fluorescence quantitative PCR
Sediri/2011 ¹⁰	Tunisia	Case-control	687(326/361)	Cases: 326/0 Controls: 361/0	Cases: 53.8 Controls: 51.1	MI	Sst I	26 6	53	7	31 5	45	1	Yes	PCR-RFLP
Abd El-Aziz/2011 ¹²	Egypt	Case-control	300(200/100)	Cases: 67/33 Controls: 32/18	Cases: 52.9 Controls: 50.7	MI	Sst I	15 0	42	8	70	10	20	Yes	PCR-RFLP
AshokKumar/2010 ¹⁵	India	Case-control	832(416/416)	Cases: 322/94 Controls: 315/101	Cases: 53.23 Controls: 53.59	CHD	Sst I	18 9	19 3	34	21 8	176	22	Yes	PCR-RFLP

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Bhanushali/2010 ¹¹	India	Case-control	240(50/190)	Cases: 82/8 Controls: 146/44	Cases: 47 Controls: 48	CHD	Sst I	-	-	-	-	-	-	Yes	PCR-RFLP
Muendlein/2008 ¹⁶	Austria	Cross-section	557(332/225)	Cases: 264/68 Controls: 123/102	Cases: 62.5 Controls: 61.5	CHD	C-482T	16 2	14 3	27 7	11 7	87 21	21	Yes	Real-time fluorescence quantitative PCR
Martinelli/2007 ¹⁷	Italy	Case-control	913(669/244)	Cases: 544/125 Controls: 168/76	Cases: 60.7 Controls: 58.7	CHD	T-455C	24 4	30 0	12 5	97 118	29	29	Yes	Immobilized oligonucleotide probes array
Dallongeville/2006 ¹⁸	France	Case-control	917(442/475)	Cases: 442/0 Controls: 475/0	Cases: 35-64 Controls: 35-64	CHD and MI	C-482T	23 7	15 5	35 5	25 5	185 31	31	Yes	PCR-RFLP
Tobin/2004 ⁹	UK	Case-control	1054(549/505)	Cases: 372/177 Controls: 313/192	Cases: 61.9 Controls: 58.6	MI	C-482T	29 1	23 3	23 3	28 3	193	29	Yes	Immobilized oligonucleotide probes array
							T-455C	21 1	28 4	52 4	21 4	229	62	Yes	
							C1100 T	29 8	20 9	40 6	29 6	172	37	Yes	

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Liu/2004 ²⁰	USA	Nested case-control	758(385/373)	Cases: 385/0 Controls: 373/0	Cases: 60 Controls: 59	MI	Sst I	29 5	77	6	29 7	60	4	Yes	PCR-RFLP
Chhabra/2004 ²¹	India	Case-control	309(158/151)	Cases: 139/19 Controls: 139/12	Cases: 53.25 Controls: 52.45	CHD	Sst I	66	76	16	71	66	14	Yes	PCR-RFLP
Wong/2003 ²²	UK	Cohort	2808(187/2621)	Cases: 187/0 Controls: 2621/0	Cases: 56.67 Controls: 56.01	CHD	C1100T, C-428T, Sst I	-	-	-	-	-	-	-	PCR-RFLP
Izar/2003 ²³	Brazil	Case-control	224(112/112)	Cases: 65/47 Controls: 66/46	Cases: 46 Controls: 45(Median)	CHD	Sst I	81	23	3	71	32	1	Yes	PCR-RFLP
Olivieri/2002 ²⁴	Italy	Cross-section	800(549/251)	Cases: 449/100 Controls: 168/83	Cases: 60.4 Controls: 57.6	CHD	T-455C	19 4	25 3	10 2	11 0	118	23	Yes	Immobilized oligonucleotide probes array
							C1100T	29 8	20 5	46 6	12 6	108	17	Yes	
							Sst I	45 2	97	0	21 4	37	0	Yes	

Russo/2001 ²⁵	USA	Cohort	2485(20 2/2283)	Cases: 146/56 Controls: 1133/115 0	-	CHD	Sst I	-	-	-	-	-	-	Yes	PCR-RFLP
Kee/1999 ³⁰	UK	Case-co ntrol	1375(76 1/614)	Cases: 761/0 Controls: 614/0	-	MI	Sst I	50 1	11 2	1	64 5	113	3	Yes	PCR-RFLP
Wick/1995 ²⁶	Germa ny	Case-co ntrol	313(212/ 101)	-	-	CHD	Sst I	17 0	42	0	85	16	0	Yes	PCR-RFLP
Vavatsi/1995 ²⁷	Greece	Case-co ntrol	149(95/5 4)	Cases: 85/10 Controls: 46/9	Cases: 51 Controls: 50	CHD	Sst I	69	20	0	36	12	2	Yes	PCR-RFLP
Rigoli/1995 ²⁸	Italy	Case-co ntrol	124(62/6 2)	Cases: 43/19 Controls: 42/20	Cases: 58.2 Controls: 57.6	CHD	Sst I	41	21	0	52	10	0	Yes	PCR-RFLP
Miettinen/1994 ²⁹	Finlan d	Case-co ntrol	132(82/5 0)	Cases: 78/4 Controls: 42/8	Cases: 40.8 Controls: 38.7	CHD	Sst I	62	19	1	37	12	1	Yes	PCR-RFLP

MI: myocardial infarction; AMI: acute myocardial infarction; CHD: coronary heart disease; HWE: Hardy-Weinberg Equilibrium; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism

-: not reported.

Supplementary Table 2 Quality assessment of the included studies

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Quality
Yu/2011 ⁹	1	1	0	0	1	1	1	1	6
Sediri/2011 ¹⁰	0	1	0	1	1	1	1	1	6
Abd El-Aziz/2011 ¹²	0	0	1	1	2	1	1	1	7
AshokKumar/2010 ¹⁵	1	0	1	1	2	1	1	1	8
Bhanushali/2010 ¹¹	1	0	0	1	1	1	1	1	6
Muendlein/2008 ¹⁶	1	1	0	0	1	1	1	1	7
Martinelli/2007 ¹⁷	1	1	0	1	1	1	1	1	7
Dallongeville/2006 ¹⁸	1	0	0	0	1	1	1	1	5
Tobin/2004 ¹⁹	1	1	1	1	1	1	1	1	8
Liu/2004 ²⁰	0	1	1	1	1	1	1	1	7
Chhabra/2004 ²¹	1	0	0	1	1	1	1	1	6
Wong/2003 ²²	1	1	1	0	1	1	1	1	7
Izar/2003 ²³	1	1	1	1	2	1	1	1	9
Olivieri/2002 ²⁴	1	0	0	1	0	1	1	1	5
Russo/2001 ²⁵	1	1	1	1	1	1	1	0	7
Kee/1999 ³⁰	1	1	0	1	1	1	1	0	6
Wick/1995 ²⁶	1	0	0	1	0	1	1	1	5
Vavatsi/1995 ²⁷	1	0	0	1	1	1	1	1	6
Rigoli/1995 ²⁸	1	0	0	1	2	1	1	1	7
Miettinen/199 ²⁹	1	1	1	1	1	1	1	1	8

For case-control and cross-sectional studies

Q1: Is the case definition adequate?

- a) yes, with independent validation b) yes, eg record linkage or based on self-reports c) no description

Q2: Representativeness of the cases

- a) consecutive or obviously representative series of cases b) potential for selection biases or not stated

Q3: Selection of Controls

- a) community controls b) hospital controls c) no description

Q4: Definition of Controls

- a) no history of disease (endpoint) b) no description of source

Q5: Comparability of cases and controls on the basis of the design or analysis

- a) study controls for age b) study controls for any additional factor

Q6: Ascertainment of exposure

- a) secure record b) structured interview where blind to case/control status
c) interview not blinded to case/control status d) written self-report or medical record only
e) no description

Q7: Same method of ascertainment for cases and controls

- a) yes b) no

Q8: Non-Response rate

- a) same rate for both groups b) non respondents described c) rate different and no designation

For cohort studies

Q1: Representativeness of the exposed cohort

- a) truly representative of the average population in the community b) somewhat representative of the average population in the community
c) selected group of users d) no description of the derivation of the cohort

Q2: Selection of the non exposed cohort

- a) drawn from the same community as the exposed cohort b) drawn from a different source

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- c) no description of the derivation of the non-exposed cohort
- Q3: Ascertainment of exposure
 - a) secure record
 - b) structured interview
 - c) written self-report
 - d) no description
- Q4: Demonstration that outcome of interest was not present at start of study
 - a) yes
 - b) no
- Q5: Comparability of cohorts on the basis of the design or analysis
 - a) study controls for age
 - b) study controls for any additional factor Outcome
- Q6: Assessment of outcome
 - a) independent blind assessment
 - b) record linkage
 - c) self-report
 - d) no description
- Q7: Was follow-up long enough for outcomes to occur
 - a) yes
 - b) no
- Q8: Adequacy of follow up of cohorts
 - a) complete follow up - all subjects accounted for
 - b) subjects lost to follow up unlikely to introduce bias - small number lost > 70 % follow up, or description provided of those lost
 - c) follow up rate < 70% and no description of those lost
 - d) no statement

Supplementary Table 3 Studies with multivariable OR

Study	Polymorphism	Univariate OR (95% CI)	Multivariable OR (95% CI)	Adjusted factors
Sediri/2011 ¹⁰	Sst I	1.54 (1.02-2.34)	2.02 (1.11-3.67)	age, diabetes, dyslipidemia, BMI and smoking
Muendlein/2008 ¹⁶	C-482T	1.14 (0.81-1.60)	1.18 (0.8-1.75)	age, sex, T2DM, BMI, hypertension, and smoking
Martinelli/2007 ¹⁷	T-455C	1.15 (0.85-1.55)	1.82 (1.05-3.18)	age, sex, smoke, hypertension, diabetes, BMI,

				creatinine, LDL-cholesterol, HDL-cholesterol, TG, ApoC-III and hs-CRP
Dallongeville/2006 ¹⁸	C-482T	0.95 (0.73-1.23)	0.91 (0.69-1.22)	age
Liu/2004 ²⁰	Sst I	1.13 (0.80-1.61)	1.25 (0.74–2.10)	age, cigarette smoking, BMI, alcohol intake, physical activity, history of diabetes mellitus, history of high cholesterol, history of hypertension, and use of multivitamins
Wong/2003 ²²	Sst I	0.72 (0.45-1.15)	0.70 (0.44-1.12)	age and practice and triglyceride levels
Olivieri/2002 ²⁴	Sst I	1.24 (0.82-1.87)	0.97 (0.59–1.59)	age, gender, smoking status, presence of diabetes and hypertension, cholesterol, triglycerides, apoA-I, and apoB.

BMI: body mass index; T2DM: type 2 diabetes mellitus; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglyceride; hs-CRP: high sensitivity C-reaction protein.

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Checklist PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			

Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7-10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7-10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097