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

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(Running title: ApoC3 polymorphisms and CHD risk)

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We declare that we have no conflict of interest.

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 boderline 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

CHD risk significantly.

Key Words: coronary heart disease, apolipoprotein C-III, genetic polymorphisms, risk factors, meta-analysis.

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 significantly.

Strengths and limitations of the study

The present study included twenty-one studies comprising 15591 subjects and comprehensively evaluated the association between ApoC3 polymorphisms and CHD risk.

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 CHD[1]. 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 E[4]. Therefore, high levels of ApoC3 may cause hypertriglyceridemia[5]. 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 factors[8]. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism[9-12]. In recent years, studies have investigated the correction 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

infarction" OR "acute coronary syndrome" OR "ischemic heart disease" OR "cardiovascular disease" OR "CHD" OR "CAD" OR "MI" OR "ACS" OR "IHD"; "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 statement[13].

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

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).

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. Under dominant model (S1S2+S2S2 vs. S1S1), the pooled OR of all studies was 1.19 (95% CI 1.00-1.42) (Figure 2, Table 1), indicating a borderline significant association between Sst I polymorphism and CHD risk. There was significant heterogeneity among studies (I^2 = 48.9%, p=0.017) (Figure 2, Table 1).

According to study characteristics, subgroup analysis was adopeted, as shown in Table 2. Pooled resluts showed that S1S2 and S2S2 genotyoes might increase risk of CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence the result, Sst I polymorphism was significantly associated with CHD risk in retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35).

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

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 atherosclerosis[30]. Clinical research found that ApoC3 levels were predictor of risk for development of CHD[31-33]. In the present study, twenty-one studies were included and four polymorphisms of ApoC3 were evaluated, including C-482T, T-455C, Sst I and C1100T polymorphism. 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 significant association was observed between Sst Ipolymorphism and CHD risk. While no evidence suggested significant association between C-482T and C1100T polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism, and we found that Sst I polymorphism was significantly associated with MI risk. Besides, Sst I polymorphism was significantly associated with CHD risk in Asian population but not in Caucasian population, indicating that the effect of Sst I polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was indicated to be significantly associated with CHD risk but this association was not confirmed in prospective studies. So, the association between Sst I polymorphism and CHD risk should be interpreted cautiously.

The mechanism of Sst I polymorphism in CHD susceptibility may be multiple. Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is possible that this polymorphism is in linkage disequilibrium with other functional polymorphism in the nearby region, such as T-455C polymorphism[10]. Sst I

polymorphism might alter plasma lipid concentrations. Several studies showed S2 carriers have higher plasma total cholesterol, TG and LDL-C levels[12 34 35], though other studies did not demonstrate significant difference[10 19 36]. Besides, it has been shown that S2 allele might significantly influence dyslipidemic state and atherosclerosis severity when patients changed their diet from saturated fatty acids to olive oil[37]. So, Sst I polymorphism plays an important role in modulating lipid levels response to dietary changes.

T-455C and C-482T polymorphisms both located in the 5' promoter region and were in strong linkage disequilibrium with each other. These two polymorphisms have been studied extensively because they could alter the nuclear transcript factors which mediate the insulin response. Significant association between T-455C polymorphism and risk of CHD was found under dominant model. For C-482T polymorphism, no significant association with CHD risk was found.

Different mechanisms could be linked to this finding. In previous works, T-455C polymorphism was associated with increased TG and ApoC3 levels[23 38]. Also, T-455C polymorphism was demonstrated to be significantly interacted with metabolic syndrome[38]. Another study showed T-455C polymorphism could interfere with n-3 polyunsaturated fatty acids on ApoC3 concentrations[7]. They found that CC homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3 polyunsaturated fatty acids[7].

The present meta-analysis has several strengths. First, our study included twenty-one studies comprising 15591 subjects, which was sufficient to allow adequate

statistical power to calculate the results. Second, we comprehensively evaluated the association between ApoC3 polymorphisms and CHD risk, and a total of four polymorphisms of ApoC3 were assessed. Besides, no publication bias was observed, indicating the pooled results might be unbiased.

The current analysis also has several limitations. First, most of the included studies investigated Asian or Caucasian population, so the conclusions may not be true for other ethnic groups. Second, significant heterogeneity was found and could not be completely explained when assess some polymorphisms. Finally, only articles published in English were included.

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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Data sharing statement: No additional data are available.

We declare that we have no conflict of interest.

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.

Funding Statement:

Competing Interest:

No competing interest

Reference:

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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.

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

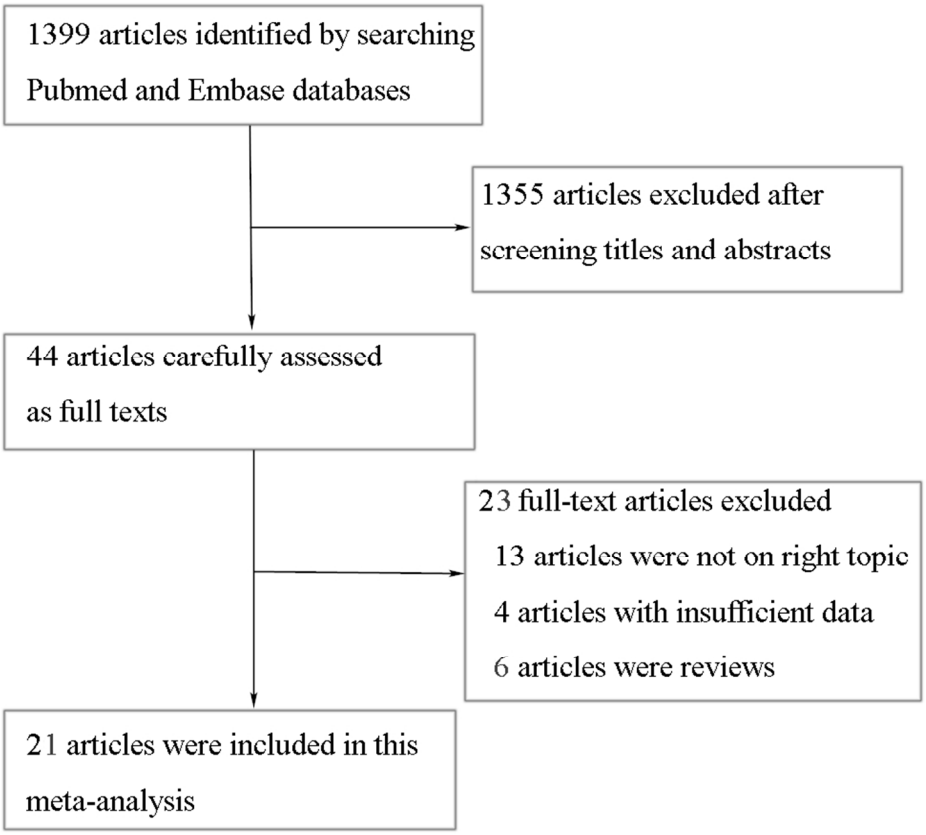

Figure 1.Flow diagram of study selection process.
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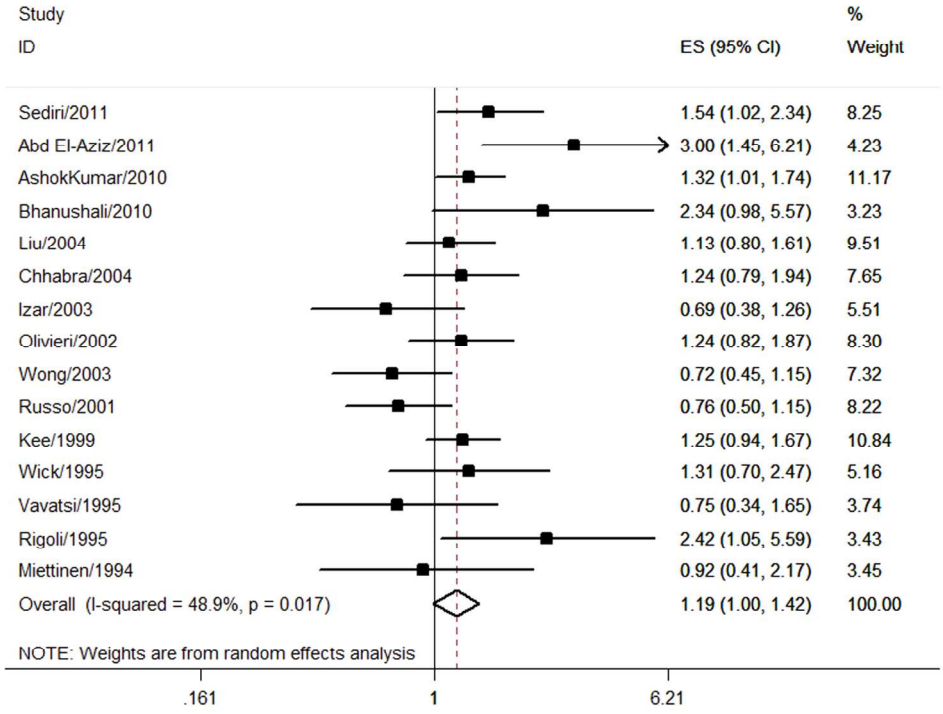

Figure 2. Association between Sst I polymorphism and CHD risk.
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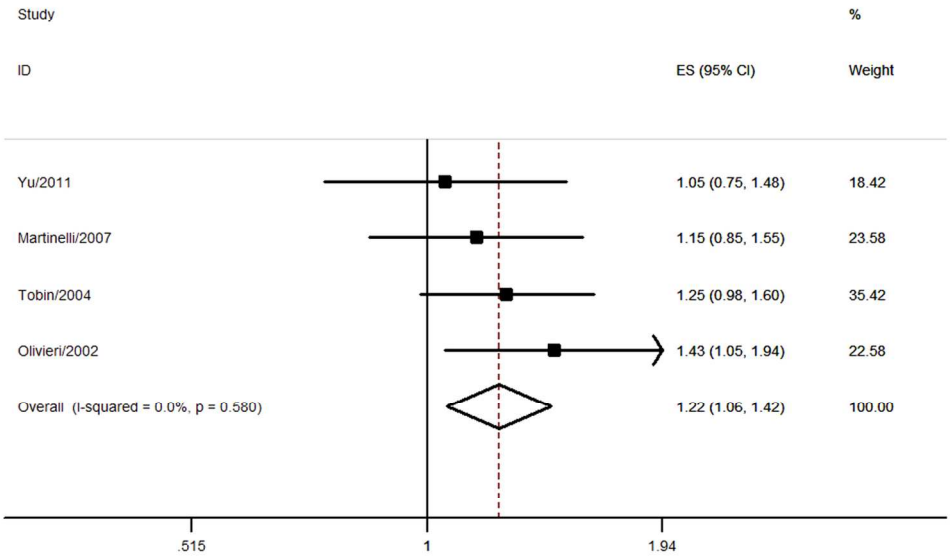

Figure 3. Association between T-455C polymorphism and CHD risk.
127x84mm (300 x 300 DPI)

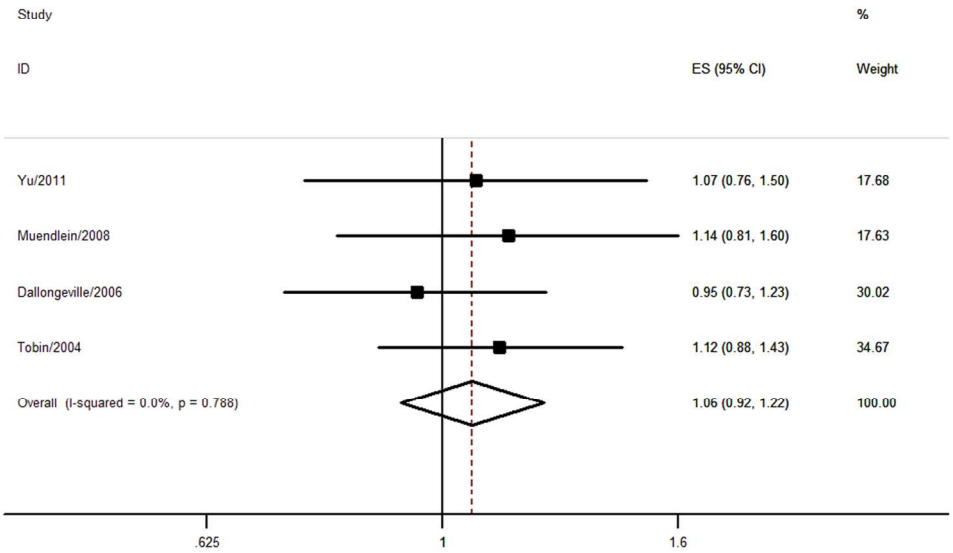

Figure 4. Association between C-482T polymorphism and CHD risk.
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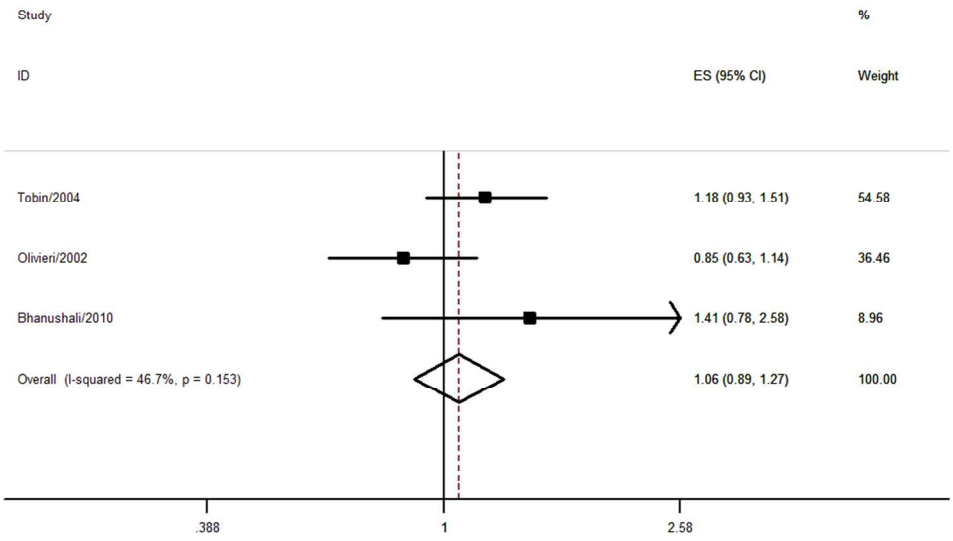

Figure 5. Association between C1100T polymorphism and CHD risk.
127x82mm (300 x 300 DPI)

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
								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	90	131	47	112	157	54	Yes
							C-482T	89	131	48	112	159	52	Yes
Sediri/2011[10]	Tunisia	Case-control	687(326/361)	Cases: 326/0 Controls: 361/0	Cases: 53.8 Controls: 51.1	MI	Sst I	266	53	7	315	45	1	Yes
Abd El-Aziz/2011[12]	Egypt	Case-control	300(200/100)	Cases: 67/33 Controls: 32/18	Cases: 52.9 Controls: 50.7	AMI	Sst I	150	42	8	70	10	20	Yes
AshokKumar/2010[14]	India	Case-control	832(416/416)	Cases: 322/94 Controls: 315/101	Cases: 53.23 Controls: 53.59	CHD	Sst I	189	193	34	218	176	22	Yes
Bhanushali/	India	Case-contr	240(50/1	Cases:	Cases:	CHD	Sst I and	-	-	-	-	-	-	-

2010[11]		ol	90)	82/8 Controls: 146/44	47 Controls: 48		C1100T							
Muendlein/2008[15]	Austria	Cross-section	557(332/225)	Cases: 264/68 Controls: 123/102	Cases: 62.5 Controls: 61.5	CHD	C-482T	162	143	27	117	87	21	Yes
Martinelli/2007[16]	Italy	Case-control	913(669/244)	Cases: 544/125 Controls: 168/76	Cases: 60.7 Controls: 58.7	CHD	T-455C	244	300	125	97	118	29	Yes
Dallongeville/2006[17]	France	Case-control	917(442/475)	Cases: 442/0 Controls: 475/0	Cases: 35-64 Controls: 35-64	CHD and MI	C-482T	237	155	35	255	185	31	Yes
Olivieri/2005[7]	Italy	Cross-section	848(590/258)	Cases: 486/104 Controls: 165/93	Cases: 60.5 Controls: 58.3	CHD	T-455C	212	271	107	116	118	24	Yes
Tobin/2004[18]	UK	Case-control	1054(549/505)	Cases: 372/177 Controls: 313/192	Cases: 61.9 Controls: 58.6	MI	C-482T	291	233	23	283	193	29	Yes
							T-455C	211	284	52	214	229	62	Yes
							C1100T	29	20	40	29	17	37	Yes

								8	9		6	2		
Liu/2004[19]	USA	Nested case-control	758(385/373)	Cases: 385/0 Controls: 373/0	Cases: 60 Controls: 59	MI	Sst I	295	77	6	297	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	194	253	102	110	118	23	Yes
							C1100T	298	205	46	126	108	17	Yes
							Sst I	452	97	0	214	37	0	Yes
Russo/2001[2]	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 Controls: 614/0	-	MI	Sst I	501	112	1	645	113	3	Yes
Wick/1995[25]	Germany	Case-control	313(212/101)	-	-	CHD	Sst I	170	42	0	85	16	0	Yes
Vavatsi/1995[26]	Greece	Case-control	149(95/54)	Cases: 85/10 Controls: 46/9	Cases: 51 Controls: 50	CHD	Sst I	69	20	0	36	12	2	Yes
Rigoli/1995[27]	Italy	Case-control	124(62/62)	Cases: 43/19 Controls: 42/20	Cases: 58.2 Controls: 57.6	CHD	Sst I	41	21	0	52	10	0	Yes
Miettinen/1994[28]	Finland	Case-control	132(82/50)	Cases: 78/4 Controls: 42/8	Cases: 40.8 Controls: 38.7	CHD	Sst I	62	19	1	37	12	1	Yes

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

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.	

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

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(Running title: ApoC3 polymorphisms and CHD risk)

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We declare that we have no conflict of interest.

Key Words: coronary heart disease, apolipoprotein C-III, genetic polymorphisms, risk factors, meta-analysis

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 boderline 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 polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42). A borderline significant association was suggested between T-455C polymorphism and MI risk (pooled OR=1.21, 95% CI 1.00-1.46). C-482T and C1100T polymorphisms were not indicated to be associated with CHD risk or MI risk.

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

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 CHD[1]. 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 E[4]. Therefore, high levels of ApoC3 may cause hypertriglyceridemia[5]. 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 factors[8]. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism[9-12]. 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 statement[13].

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

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, whether the polymorphism(s) evaluated was in Hardy-Weinberg equilibrium or not, and the genotyping assay method. Any discrepancy was resolved by a third investigator. Newcastle-Ottawa Scale (NOS) method was applied to assess the study quality[14]. The NOS contains eight items and the score ranged from 0 to 9.

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. Study-specific ORs of CHD were firstly pooled and then we evaluated the association between ApoC3 polymorphisms and MI risk separately. 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 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 not[22]. Most of the study used restriction fragment length polymorphism

(RFLP) method for DNA genotyping (n=14) and one study applied immobilized oligonucleotide probes array (IOPA) method[24]. Multivariable OR could be extracted from 4 studies[10 20 22 24] (Supplementary Table 3). Under dominant model (S1S2+S2S2 vs. S1S1), the pooled univariate OR of all studies was 1.19 (95% CI 1.00-1.42) (Figure 2, Table 1), indicating a borderline significant association between Sst I polymorphism and CHD risk. There was significant heterogeneity among studies (I^2 = 48.9%, p=0.017) (Figure 2, Table 1). The pooled multivariable OR was 1.11 (0.73-1.70), did not suggest a significant association.

According to study characteristics, subgroup analysis was adopted, as shown in Table 2. Pooled results showed that S1S2 and S2S2 genotyes might increase risk of CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence the result, Sst I polymorphism was significantly associated with CHD risk in retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35). After excluding the study did not report Hardy-Weinberg equilibrium, the pooled OR was 1.24 (95% CI 1.04-1.47). The pooled OR of the studies applied RFLP method was 1.19 (95% CI 0.98-1.44).

Meta-analysis of T-455C polymorphism

The association between T-455C polymorphism and CHD risk was evaluated by 4

studies comprising 3378 individuals. All the studies were in Hardy-Weinberg equilibrium. Immobilized oligonucleotide probes array method was used by three studies while real-time fluorescence quantitative PCR was applied by one study[9]. Only one study reported a multivariable OR of 1.82 (95% CI 1.05-3.18), while the unadjusted OR was 1.15 (98% CI 0.85-1.55)[17] (Supplementary Table 3). 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). Two studies reported the association between T-455C polymorphism and MI risk, and the pooled result suggested a borderline significant association (pooled OR=1.21, 95% CI 1.00-1.46).

Meta-analysis of C-482T polymorphism

Four studies with 3070 individuals reported the association between C-482T polymorphism and CHD risk. Only one study did not report whether C-482T polymorphism was in Hardy-Weinberg equilibrium or not[22]. Two studies applied real-time fluorescence quantitative PCR method[9 16], one study used RFLP method[22] and the other study adopted immobilized oligonucleotide probes array method[19]. Multivariable OR could be extracted from two studies[16 18] (Supplementary Table 3). 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). Only one study reported the association between C-482T polymorphism and MI risk

(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 method[22]. 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 atherosclerosis[31]. Clinical research found that ApoC3 levels were predictor of risk for development of CHD[32-34]. 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

significant association was observed between Sst Ipolymorphism and CHD risk. While no evidence suggested significant association between C-482T and C1100T polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism, and we found that Sst I polymorphism was significantly associated with MI risk. Besides, Sst I polymorphism was significantly associated with CHD risk in Asian population but not in Caucasian population, indicating that the effect of Sst I polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was indicated to be significantly associated with CHD risk but this association was not confirmed in prospective studies. So, the association between Sst I polymorphism and CHD risk should be interpreted cautiously.

The mechanism of Sst I polymorphism in CHD susceptibility may be multiple. Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is possible that this polymorphism is in linkage disequilibrium with other functional polymorphism in the nearby region, such as T-455C polymorphism[10]. Sst I polymorphism might alter plasma lipid concentrations. Several studies showed S2 carriers have higher plasma total cholesterol, TG and LDL-C levels[12 35 36], though other studies did not demonstrate significant difference[10 20 37]. Besides, it has been shown that S2 allele might significantly influence dyslipidemic state and atherosclerosis severity when patients changed their diet from saturated fatty acids to olive oil[38]. So, Sst I polymorphism plays an important role in modulating lipid levels response to dietary changes.

T-455C and C-482T polymorphisms both located in the 5' promoter region and

were in strong linkage disequilibrium with each other. These two polymorphisms have been studied extensively because they could alter the nuclear transcript factors which mediate the insulin response. Significant association between T-455C polymorphism and risk of CHD was found under dominant model. However, it should be noted that in the 4 studies evaluating T-455C polymorphism, only one study[24] showed a significant association between T-455C polymorphism and CHD risk. So, more studies with large sample size are warranted to clarify this issue. For C-482T polymorphism, no significant association with CHD risk was found.

Different mechanisms could be linked to this finding. In previous works, T-455C polymorphism was associated with increased TG and ApoC3 levels[24 39]. Also, T-455C polymorphism was demonstrated to be significantly associated with metabolic syndrome[39]. Another study showed T-455C polymorphism could interfere with n-3 polyunsaturated fatty acids on ApoC3 concentrations[7]. Olivieri O *et al.* found that CC homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3 polyunsaturated fatty acids[7].

The present meta-analysis has several strengths. First, this study was based on guidelines for conducting and reporting systematic reviews and the methods were rigorous. Second, we comprehensively evaluated the association between ApoC3 polymorphisms and CHD risk, and a total of four polymorphisms of ApoC3 were assessed. Besides, no publication bias was observed, indicating the pooled results might be unbiased.

The current analysis also has several limitations. First, most of the included studies

investigated Asian or Caucasian population, so the conclusions may not be true for other ethnic groups. Second, significant heterogeneity was found and could not be completely explained when assess some polymorphisms. Third, only Pubmed, Embase and Cochrane library were searched for eligible articles. Finally, only articles published in English were included.

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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Contributorship: 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.

Data sharing statement: No additional data are available.

Competing: 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.

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

Supplementary Table 2 Quality assessment of the included studies

Supplementary Table 3 Studies reporting multivariable OR

Checklist PRISMA checklist

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

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We declare that we have no conflict of interest.

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 boderline 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

polymorphism and CHD risk (TC+CC vs. TT, pooled OR=1.22, 95% CI 1.06-1.42). A borderline significant association was suggested between T-455C polymorphism and MI risk (pooled OR=1.21, 95% CI 1.00-1.46). C-482T and C1100T polymorphisms were not indicated to be associated with CHD risk or MI risk.

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

Key Words: coronary heart disease, apolipoprotein C-III, genetic polymorphisms, risk factors, meta-analysis.

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 CHD[1]. 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 E[4]. Therefore, high levels of ApoC3 may cause hypertriglyceridemia[5]. 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 factors[8]. Several polymorphisms have been found in ApoC3 gene, including C-482T, T-455C, Sst I, C1100T polymorphism[9-12]. 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 statement[13].

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 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, whether the polymorphism(s) evaluated was

in Hardy-Weinberg equilibrium or not, and the genotyping assay method. Any discrepancy was resolved by a third investigator. Newcastle-Ottawa Scale (NOS) method was applied to assess the study quality[14]. The NOS contains eight items and the score ranged from 0 to 9.

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. Study-specific ORs of CHD were firstly pooled and then we evaluated the association between ApoC3 polymorphisms and MI risk separately. 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 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 not[22]. Most of the study used restriction fragment length polymorphism (RFLP) method for DNA genotyping (n=14) and one study applied immobilized oligonucleotide probes array (IOPA) method[24]. Multivariable OR could be extracted from 4 studies[10 20 22 24] (Supplementary Table 3). Under dominant model (S1S2+S2S2 vs. S1S1), the pooled univariate OR of all studies was 1.19 (95% CI 1.00-1.42) (Figure 2, Table 1), indicating a borderline significant association between Sst I

polymorphism and CHD risk. There was significant heterogeneity among studies (I^2 = 48.9%, p=0.017) (Figure 2, Table 1). The pooled multivariable OR was 1.11 (0.73-1.70), did not suggest a significant association.

According to study characteristics, subgroup analysis was adopted, as shown in Table 2. Pooled results showed that S1S2 and S2S2 genotyes might increase risk of CHD in Asian population (pooled OR=1.35, 95% CI 1.08-1.69) but not in Caucasian population (pooled OR=1.14, 95% CI 0.92-1.41). Study design could also influence the result, Sst I polymorphism was significantly associated with CHD risk in retrospective studies (pooled OR=1.30, 95% CI 1.04-1.61) but not in prospective studies (pooled OR=0.98, 95% CI 0.75-1.28). Besides, Sst I polymorphism was observed to be significantly associated with MI risk (pooled OR=1.42, 95% CI 1.06-1.91) but not CHD risk (pooled OR=1.09, 95% CI 0.87-1.35). After excluding the study did not report Hardy-Weinberg equilibrium, the pooled OR was 1.24 (95% CI 1.04-1.47). The pooled OR of the studies applied RFLP method was 1.19 (95% CI 0.98-1.44).

Meta-analysis of T-455C polymorphism

The association between T-455C polymorphism and CHD risk was evaluated by 4 studies comprising 3378 individuals. All the studies were in Hardy-Weinberg equilibrium. Immobilized oligonucleotide probes array method was used by three studies while real-time fluorescence quantitative PCR was applied by one study[9]. Only one study reported a multivariable OR of 1.82 (95% CI 1.05-3.18), while the unadjusted OR was 1.15 (98% CI 0.85-1.55)[17] (Supplementary Table 3). 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). Two studies reported the association between T-455C polymorphism and MI risk, and the pooled result suggested a borderline significant association (pooled OR=1.21, 95% CI 1.00-1.46).

Meta-analysis of C-482T polymorphism

Four studies with 3070 individuals reported the association between C-482T polymorphism and CHD risk. Only one study did not report whether C-482T polymorphism was in Hardy-Weinberg equilibrium or not[22]. Two studies applied real-time fluorescence quantitative PCR method[9 16], one study used RFLP method[22] and the other study adopted immobilized oligonucleotide probes array method[19]. Multivariable OR could be extracted from two studies[16 18] (Supplementary Table 3). 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). Only one study reported the association between C-482T polymorphism and MI risk (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 method[22]. 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 atherosclerosis[31]. Clinical research found that ApoC3 levels were predictor of risk for development of CHD[32-34]. 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 significant association was observed between Sst Ipolymorphism and CHD risk. While no evidence suggested significant association between C-482T and C1100T polymorphisms and CHD risk. Subgroup analysis was applied for Sst I polymorphism, and we found that Sst I polymorphism was significantly associated with MI risk. Besides, Sst I polymorphism was significantly associated with CHD risk in Asian

population but not in Caucasian population, indicating that the effect of Sst I polymorphism might be influenced by ethnicity. For retrospective studies, Sst I was indicated to be significantly associated with CHD risk but this association was not confirmed in prospective studies. So, the association between Sst I polymorphism and CHD risk should be interpreted cautiously.

The mechanism of Sst I polymorphism in CHD susceptibility may be multiple. Sst I polymorphism is located in 3' untranslated region of the ApoC3 gene, and it is possible that this polymorphism is in linkage disequilibrium with other functional polymorphism in the nearby region, such as T-455C polymorphism[10]. Sst I polymorphism might alter plasma lipid concentrations. Several studies showed S2 carriers have higher plasma total cholesterol, TG and LDL-C levels[12 35 36], though other studies did not demonstrate significant difference[10 20 37]. Besides, it has been shown that S2 allele might significantly influence dyslipidemic state and atherosclerosis severity when patients changed their diet from saturated fatty acids to olive oil[38]. So, Sst I polymorphism plays an important role in modulating lipid levels response to dietary changes.

T-455C and C-482T polymorphisms both located in the 5' promoter region and were in strong linkage disequilibrium with each other. These two polymorphisms have been studied extensively because they could alter the nuclear transcript factors which mediate the insulin response. Significant association between T-455C polymorphism and risk of CHD was found under dominant model. However, it should be noted that in the 4 studies evaluating T-455C polymorphism, only one study[24]

showed a significant association between T-455C polymorphism and CHD risk. So, more studies with large sample size are warranted to clarify this issue. For C-482T polymorphism, no significant association with CHD risk was found.

Different mechanisms could be linked to this finding. In previous works, T-455C polymorphism was associated with increased TG and ApoC3 levels[24 39]. Also, T-455C polymorphism was demonstrated to be significantly associated with metabolic syndrome[39]. Another study showed T-455C polymorphism could interfere with n-3 polyunsaturated fatty acids on ApoC3 concentrations[7]. Olivieri O *et al.* found that CC homozygous carriers were poorly responsive to the ApoC3 lowering effects of n-3 polyunsaturated fatty acids[7].

The present meta-analysis has several strengths. First, this study was based on guidelines for conducting and reporting systematic reviews and the methods were rigorous. Second, we comprehensively evaluated the association between ApoC3 polymorphisms and CHD risk, and a total of four polymorphisms of ApoC3 were assessed. Besides, no publication bias was observed, indicating the pooled results might be unbiased.

The current analysis also has several limitations. First, most of the included studies investigated Asian or Caucasian population, so the conclusions may not be true for other ethnic groups. Second, significant heterogeneity was found and could not be completely explained when assess some polymorphisms. Third, only Pubmed, Embase and Cochrane library were searched for eligible articles. Finally, only articles published in English were included.

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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the manuscript.

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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.

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

Supplementary Table 2 Quality assessment of the included studies

Supplementary Table 3 Studies reporting multivariable OR

Checklist PRISMA checklist

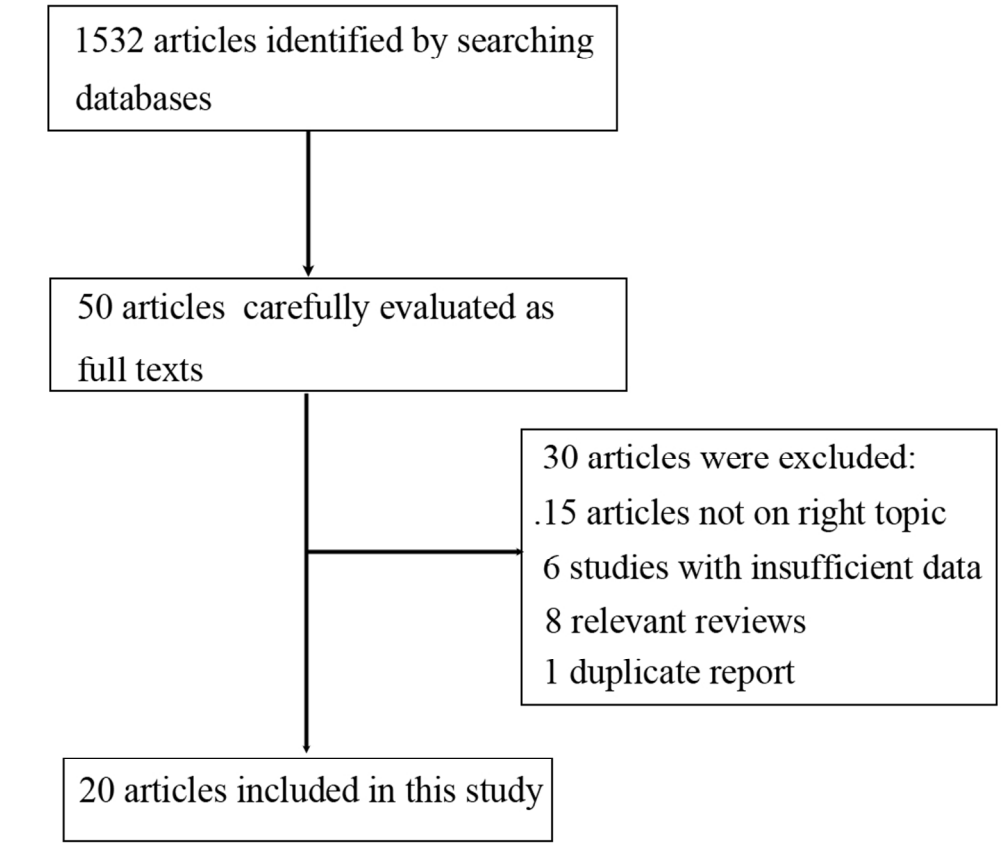

Figure 1.Flow diagram of study selection process.
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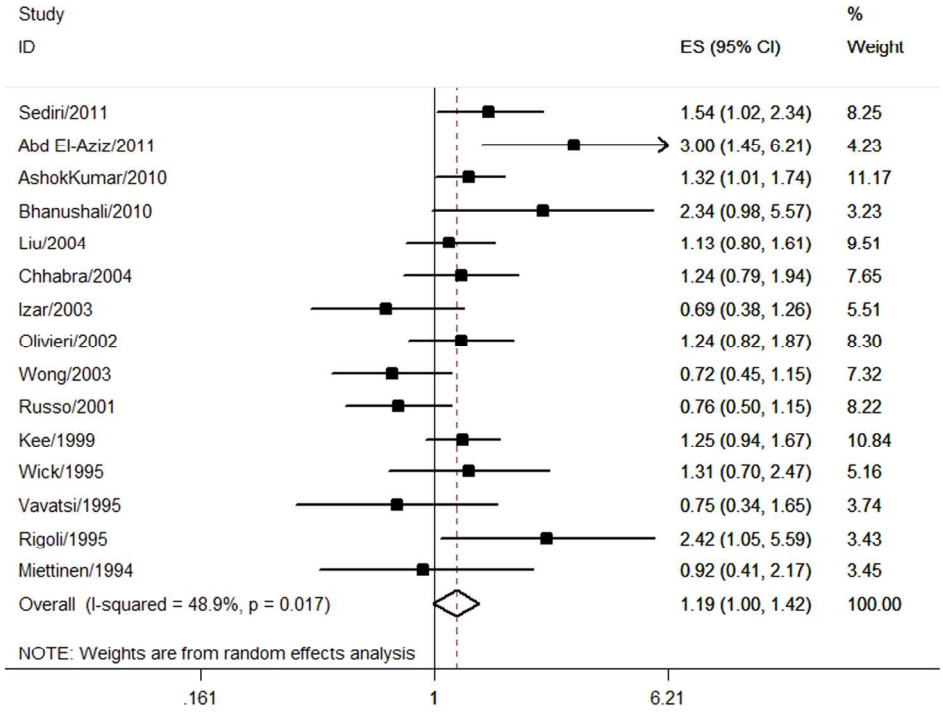

Figure 2. Association between Sst I polymorphism and CHD risk.
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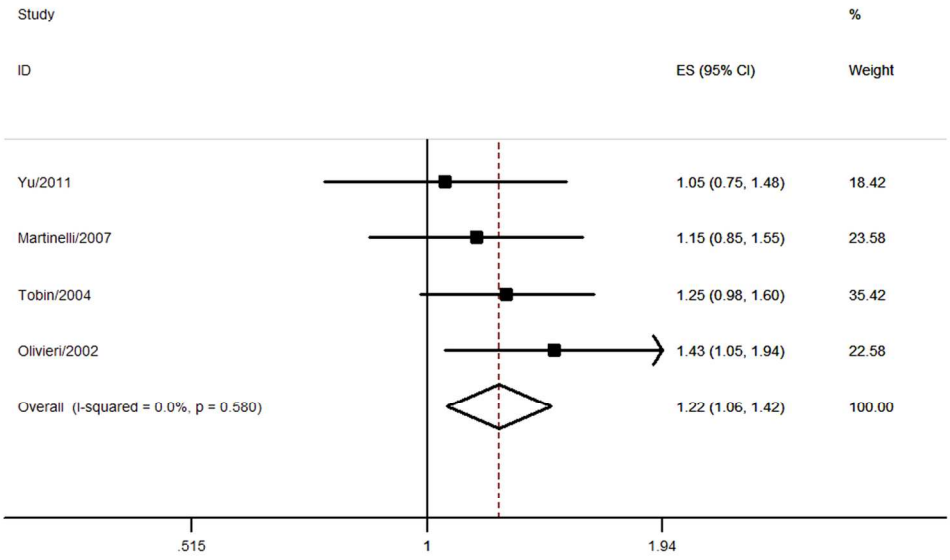

Figure 3. Association between T-455C polymorphism and CHD risk.
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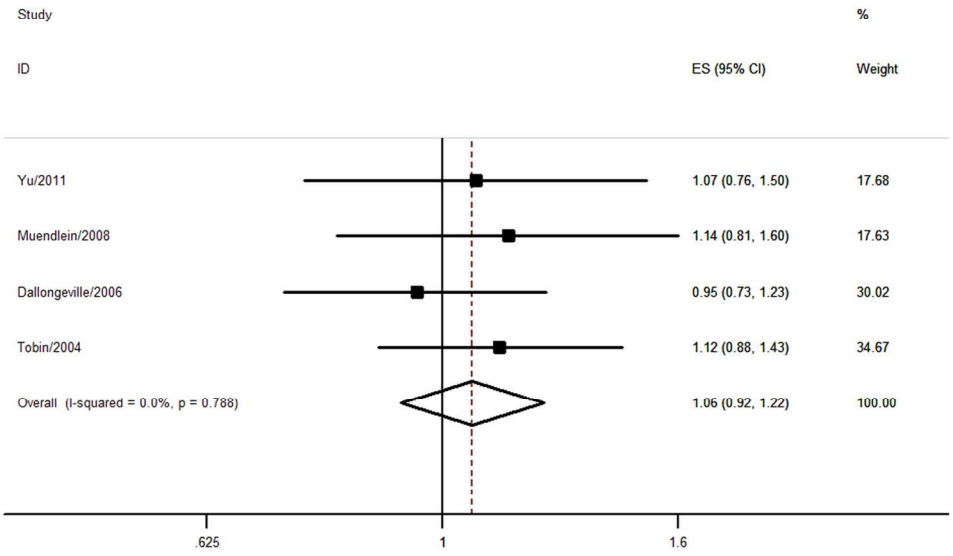

Figure 4. Association between C-482T polymorphism and CHD risk.
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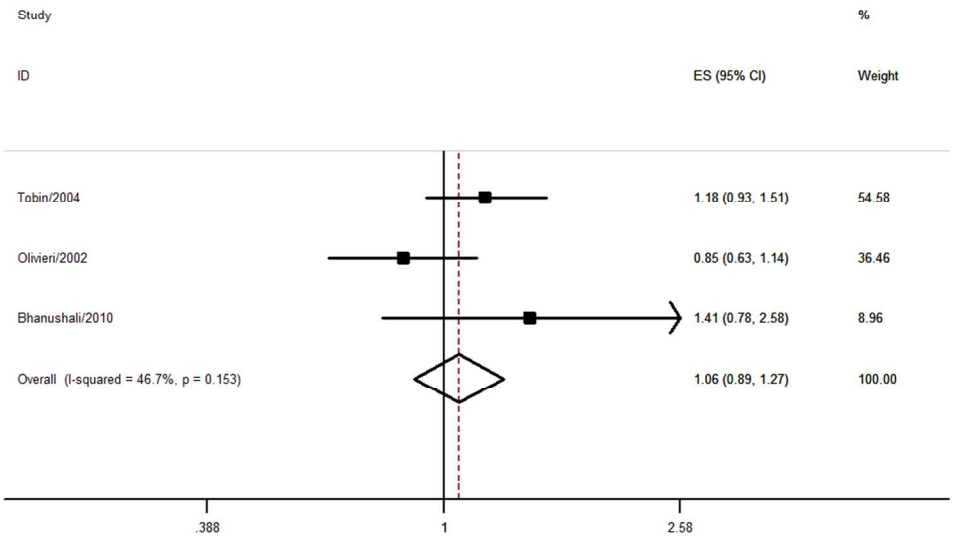

Figure 5. Association between C1100T polymorphism and CHD risk.
127x82mm (300 x 300 DPI)

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	90	131	47	112	157	54	Yes	Real-time fluorescence quantitative PCR
							C-482T	89	131	48	112	159	52	Yes	
Sediri/2011[10]	Tunisia	Case-control	687(326/361)	Cases: 326/0 Controls: 361/0	Cases: 53.8 Controls: 51.1	MI	Sst I	266	53	7	315	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	150	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	189	193	34	218	176	22	Yes	PCR-RFLP

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	162	143	27	117	87	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	244	300	125	97	118	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	237	155	35	255	185	31	Yes	PCR-RFLP
Tobin/2004[19]	UK	Case-control	1054(549/505)	Cases: 372/177 Controls: 313/192	Cases: 61.9 Controls: 58.6	MI	C-482T	291	233	23	283	193	29	Yes	Immobilized oligonucleotide probes array
							T-455C	211	284	52	214	229	62	Yes	
							C1100T	298	209	40	296	172	37	Yes	

Liu/2004[20]	USA	Nested case-control	758(385/373)	Cases: 385/0 Controls: 373/0	Cases: 60 Controls: 59	MI	Sst I	295	77	6	297	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	194	253	102	110	118	23	Yes	Immobilized oligonucleotide probes array
							C1100T	298	205	460	126	108	17	Yes	
							Sst I	452	97	0	214	37	0	Yes	

Russo/2001[25]	USA	Cohort	2485(202/2283)	Cases: 146/56 Controls: 1133/1150	-	CHD	Sst I	-	-	-	-	-	-	Yes	PCR-RFLP
Kee/1999[30]	UK	Case-control	1375(761/614)	Cases: 761/0 Controls: 614/0	-	MI	Sst I	501	112	1	645	113	3	Yes	PCR-RFLP
Wick/1995[26]	Germany	Case-control	313(212/101)	-	-	CHD	Sst I	170	42	0	85	16	0	Yes	PCR-RFLP
Vavatsi/1995[27]	Greece	Case-control	149(95/54)	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-control	124(62/62)	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]	Finland	Case-control	132(82/50)	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

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.

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

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