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Associations of the *NOS3* rs1799983 polymorphism with circulating nitric oxide and lipid levels: a systematic review and meta-analysis

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ABSTRACT

Background Circulating nitric oxide (NO) and lipid levels are closely associated with coronary artery disease (CAD). It is unclear whether the rs1799983 polymorphism in endothelial nitric oxide synthase (NOS3) gene is associated with plasma levels of NO and lipids. This systematic review and meta-analysis (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) aimed to clarify the relationships between the rs1799983 polymorphism and plasma levels of NO and lipids.

Methods Sixteen studies (2702 subjects) and 59 studies (14 148 subjects) were identified for the association analyses for NO and lipids, respectively. Mean difference (MD) and 95% CI were used to estimate the effects of the rs1799983 polymorphism on plasma NO and lipid levels. The primary outcome variable was NO, and the secondary outcomes included triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).

Results Carriers of the T allele had lower levels of NO (MD $-0.27 \mu\text{mol/L}$, 95% CI -0.42 to $-0.12 \mu\text{mol/L}$, $p < 0.001$) and HDL-C (MD -0.07 mmol/L , 95% CI -0.14 to -0.00 mmol/L , $p = 0.04$), and higher levels of TC (MD 0.13 mmol/L , 95% CI 0.06 to 0.20 mmol/L , $p < 0.001$) and LDL-C (MD 0.14 mmol/L , 95% CI 0.05 to 0.22 mmol/L , $p = 0.002$) than the non-carriers. Triglyceride levels were comparable between the genotypes.

Conclusion The association between the *NOS3* rs1799983 polymorphism and CAD may be partly mediated by abnormal NO and lipid levels caused by the T allele.

INTRODUCTION

Coronary artery disease (CAD) is one of the leading causes of death in the world.¹ A number of risk factors have been identified for CAD in the past few decades. Among these risk factors, reduction in circulating nitric oxide (NO) and dyslipidaemia were widely reported with regard to their important roles in the occurrence and development of CAD.^{2,3} NO, synthesised from L-arginine by nitric oxide synthase (NOS), is a potent vasodilator and plays a pivotal role in normally functioning cardiovascular system. Dyslipidaemia is a state of abnormal amounts of lipids in the circulation, characterised by increased levels of triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and/or decreased level of high-density lipoprotein cholesterol (HDL-C). A huge number of studies

have been conducted to investigate the associations between genetic polymorphisms and the risk factors for CAD, but the results were inconsistent and inconclusive. The main reasons for the inconsistency of results are small sample size, different health status, ethnic difference, and so on.

In humans, NOS has three isoforms: neuronal NOS (NOS1), inducible NOS (NOS2) and endothelial NOS (eNOS/NOS3). Among them, NOS3 is responsible for synthesising circulating NO and plays a crucial role in the pathogenesis of CAD.^{4,5} Chen *et al*⁴ demonstrated that the mRNA and protein levels of NOS3 were significantly decreased in patients with CAD as compared with healthy subjects, and overexpression of NOS3 increased NO production and restored cardiac function after myocardial infarction in mice. In a mouse model of arterial restenosis, the anti-restenotic effect of liraglutide was completely abolished by N-omega-nitro-L-arginine methyl ester, an inhibitor of NOS3.⁵ NOS3 gene is located on the long arm of human chromosome 7 (q35.1), and it contains 27 exons and 26 introns with a total length of 24 kb. There are several polymorphisms in NOS3 gene. The rs1799983 polymorphism (also known as G894T or Glu298Asp) is located in exon 7 of NOS3 gene and formed by a transversion from guanine (G) to thymine (T). Accordingly, the 298th genetic code is changed from GAG to GAT, resulting in the replacement of glutamic acid residue with aspartic acid residue in the NOS3 polypeptide. A series of studies have investigated the associations of the rs1799983 polymorphism with plasma levels of NO and/or lipids. In some of these studies, T allele of the rs1799983 polymorphism was reported to be associated with increased levels of TG,^{6–12} TC^{8–14} and LDL-C,^{7,9–16} and/or decreased levels of HDL-C^{10,15,17,18} and NO.^{10,19–22} However, the results from other studies did not support these findings.^{23–27} Hence, a meta-analysis is required to clarify the relationships of the rs1799983 polymorphism with NO and lipids.

In this study, a systematic review and meta-analysis was performed based on previous publications to investigate the associations of the rs1799983 polymorphism with NO and lipid levels. The results from the present meta-analysis can provide an opportunity to unveil the inter-relationships among the rs1799983 polymorphism, NO, dyslipidaemia and susceptibility to CAD.

MATERIALS AND METHODS

Search strategy

This study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (online supplementary table S1). The articles published before June 2018 on the associations between the rs1799983 polymorphism and circulating levels of NO and/or lipids were identified. The languages of the articles were limited to English and Chinese. A comprehensive search was conducted to identify all relevant articles. The following electronic databases were searched: PubMed, Embase, Baidu Scholar, Google Scholar, Web of Science, Cochrane Library, Wanfang, CBM and CNKI. The keywords used are as follows: ('endothelial nitric oxide synthase' or 'NOS3'), ('polymorphism' or 'mutation' or 'variant' or 'G894T' or 'rs1799983' or 'Glu298Asp'), ('NO' or 'nitric oxide') and ('plasma lipid' or 'blood lipid' or 'serum lipid').

Inclusion and exclusion criteria

The inclusion criteria for the meta-analysis are as follows: (1) studies which presented genotype and allele frequencies of the rs1799983 polymorphism; (2) studies in which mean NO and/or mean lipids with SD or SE according to the rs1799983 genotypes were available; (3) studies which reported at least one of the five variables, that is, NO, TG, TC, LDL-C and HDL-C. All references cited by the articles included in this meta-analysis were reviewed and checked to ensure there were no missing studies. Reports with incomplete data, studies based on pedigree data, case reports, review articles, abstracts and animal studies were excluded from the meta-analysis. Preintervention data were used for interventional studies.

Data extraction

The data were extracted using a structured data collection form as described previously.⁷⁴ Briefly, data were extracted from each study independently by two investigators according to the prespecified selection criteria. Selection decisions were compared and disagreements were resolved by consensus or involving a third investigator. For the overlapping articles, only the publications that presented the most detailed information were included. In this meta-analysis, the data extracted from each of the included studies are as follows: first author, year of publication, age, ethnicity, gender, health status, type of study, genotyping method, lipid assay method, sample size and mean with SD or SE according to the rs1799983 genotypes. If data in a study were unconvincing, we attempted to contact the corresponding or first author by email or telephone.

Statistical analysis

Due to low frequencies of the variant T allele in the studies included, a dominant model ((GT+TT) vs GG) was employed to ensure adequate statistical power. Random effects model was used to evaluate the results if heterogeneity among the included studies was significant ($I^2 > 50\%$). Otherwise, fixed effects model (inverse variance-weighted average method) was used.⁷⁵ The pooled mean difference (MD) with 95% CI was used to assess the strength of the associations between the rs1799983 polymorphism and plasma levels of NO and lipids. Heterogeneity was investigated by Cochran's χ^2 -based Q-statistic, and Galbraith plots were used to detect the potential sources of heterogeneity. MD values were recalculated after excluding the studies with heterogeneity. Subgroup analyses were performed according to gender, ethnicity and health status. Ethnic subgroup was defined as Caucasian, Asian and the subjects of other ethnic origin.

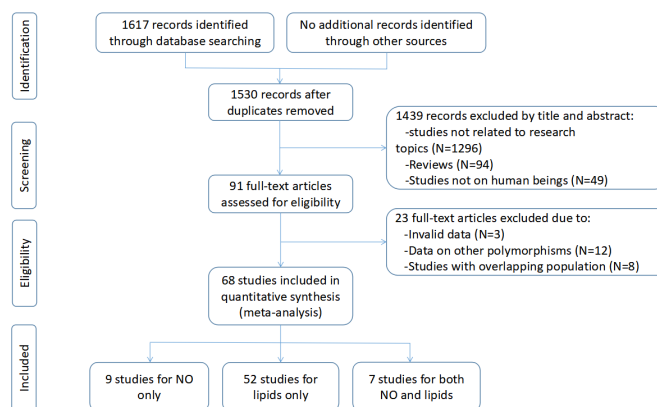


Figure 1 Flow diagram of study selection process for the systematic review and meta-analysis (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, PRISMA). NO, nitric oxide.

Health status was defined as CAD, type 2 diabetes mellitus (T2DM) and hypertension. Hardy-Weinberg equilibrium (HWE) was assessed by Fisher's exact test. MD values were recalculated after excluding the studies which were not in HWE. Publication bias was tested by Begg's funnel plot and Egger's test.⁷⁶ Statistical analysis was performed using STATA V.12.0 (StataCorp, College Station, TX, USA). All the tests were two sided and a p value < 0.05 for any test or model was considered to be statistically significant.

RESULTS

Characteristics of the included studies

Initial search of the databases yielded 1617 articles. Eight-seven duplicate studies were excluded. One thousand four hundred and thirty-nine studies were excluded according to the titles and abstracts. Then full-text articles were retrieved and assessed on the basis of the inclusion criteria. Twenty-three studies were ineligible for the following reasons: 3 studies presented invalid data; 12 studies presented data for other polymorphisms; and 8 studies had subjects overlapping with other publications. In the end, 68 studies were selected for this meta-analysis (figure 1). Among them, 16 studies (2702 subjects) were included in the association analysis for NO, and 59 studies (14 148 subjects) were included in the association analysis for lipids. The characteristics of the studies included in the meta-analysis are summarised in online supplementary table S2. The plasma NO and lipid levels according to the genotypes of the rs1799983 polymorphism are presented in online supplementary table S3 and S4, respectively.

Association of the rs1799983 polymorphism with NO

Carriers of the variant T allele had lower levels of NO (MD $-0.27 \mu\text{mol/L}$, 95% CI -0.42 to $-0.12 \mu\text{mol/L}$, $p < 0.001$) than the non-carriers (table 1, figure 2). When the analysis was limited to the studies in HWE, the association between the rs1799983 polymorphism and NO was also significant (MD $-0.23 \mu\text{mol/L}$, 95% CI -0.37 to $-0.10 \mu\text{mol/L}$, $p = 0.001$). Subgroup analyses stratified by the characteristics of the subjects were performed, and the results showed that the rs1799983 polymorphism was significantly associated with NO levels in females, Caucasians and the healthy subjects, but not in males, Asians and patients with CAD.

Table 1 Meta-analysis between the *NOS3* rs1799983 polymorphism and plasma levels of NO or lipids

Groups or subgroups	Comparisons (subjects)	MD (95% CI)	P _{heterogeneity}	P _{MD}
NO				
All	24 (2702)	-0.27 (-0.42 to -0.12)	<0.001	<0.001
Studies in HWE	22 (2502)	-0.23 (-0.37 to -0.10)	0.001	0.001
Male	3 (240)	-0.17 (-0.63 to 0.29)	0.07	0.46
Female	6 (443)	-0.44 (-0.88 to -0.00)	0.001	0.05
Caucasian	6 (595)	-0.31 (-0.48 to -0.15)	<0.001	<0.001
Asian	8 (1065)	-0.11 (-0.25 to 0.03)	0.85	0.11
Other ethnicity	10 (1042)	-0.33 (-0.60 to -0.06)	<0.001	0.02
CAD	4 (631)	-0.38 (-0.92 to 0.16)	<0.001	0.17
Healthy or control	11 (1332)	-0.25 (-0.45 to -0.06)	0.01	0.01
TG				
All	63 (12 174)	0.03 (-0.05 to 0.11)	<0.001	0.49
Studies in HWE	52 (9751)	-0.02 (-0.11 to 0.08)	<0.001	0.74
Male	6 (1155)	0.14 (-0.16 to 0.45)	<0.001	0.36
Female	6 (469)	-0.05 (-0.39 to 0.29)	0.02	0.77
Caucasian	29 (5707)	0.16 (0.04 to 0.28)	<0.001	0.01
Asian	20 (4435)	-0.14 (-0.31 to 0.03)	<0.001	0.10
Other ethnicity	14 (2032)	-0.03 (-0.12 to 0.06)	0.45	0.52
CAD	9 (2308)	0.14 (-0.05 to 0.33)	<0.001	0.14
T2DM	12 (1633)	-0.17 (-0.35 to 0.01)	0.01	0.06
Hypertension	8 (1458)	0.19 (-0.17 to 0.55)	<0.001	0.29
Healthy or control	24 (3345)	-0.01 (-0.16 to 0.14)	<0.001	0.89
TC				
All	68 (13 129)	0.13 (0.06 to 0.20)	<0.001	<0.001
Studies in HWE	57 (10 706)	0.07 (0.03 to 0.11)	0.14	0.01
Male	8 (1487)	0.11 (-0.03 to 0.25)	0.11	0.11
Female	5 (444)	0.08 (-0.32 to 0.48)	0.01	0.70
Caucasian	34 (6224)	0.17 (0.05 to 0.28)	<0.001	0.01
Asian	23 (5173)	0.11 (0.04 to 0.18)	0.61	0.001
Other ethnicity	11 (1732)	0.04 (-0.10 to 0.19)	0.05	0.56
CAD	10 (2008)	0.20 (0.02 to 0.39)	0.001	0.03
T2DM	9 (1480)	0.08 (-0.04 to 0.20)	0.94	0.19
Hypertension	11 (1894)	0.15 (-0.03 to 0.33)	0.001	0.10
Healthy or control	23 (3446)	0.04 (-0.04 to 0.12)	0.24	0.33
LDL-C				
All	48 (9153)	0.14 (0.05 to 0.22)	<0.001	0.002
Studies in HWE	40 (7659)	0.10 (0.05 to 0.14)	0.06	0.01
Male	8 (1497)	0.11 (-0.03 to 0.24)	0.17	0.11
Female	5 (444)	-0.01 (-0.34 to 0.32)	0.07	0.96
Caucasian	26 (4746)	0.19 (0.05 to 0.33)	<0.001	0.01
Asian	11 (2872)	0.11 (-0.01 to 0.23)	0.12	0.08
Other ethnicity	11 (1535)	0.03 (-0.07 to 0.13)	0.48	0.52
CAD	5 (1373)	0.18 (-0.04 to 0.40)	0.01	0.12

Continued

Table 1 Continued

Groups or subgroups	Comparisons (subjects)	MD (95% CI)	P _{heterogeneity}	P _{MD}
T2DM	4 (573)	0.16 (-0.02 to 0.33)	0.65	0.09
Hypertension	10 (1529)	0.17 (-0.05 to 0.38)	<0.001	0.13
Healthy or control	18 (2114)	0.05 (-0.06 to 0.16)	0.14	0.34
HDL-C				
All	57 (11 401)	-0.07 (-0.14 to -0.00)	<0.001	0.04
Studies in HWE	46 (8978)	-0.08 (-0.16 to -0.00)	<0.001	0.05
Male	7 (1457)	-0.02 (-0.15 to 0.10)	0.22	0.73
Female	7 (543)	-0.11 (-0.30 to 0.08)	0.83	0.26
Caucasian	28 (5375)	-0.03 (-0.10 to 0.04)	0.09	0.41
Asian	16 (4392)	-0.15 (-0.34 to 0.04)	<0.001	0.12
Other ethnicity	13 (1634)	-0.06 (-0.18 to 0.06)	0.22	0.32
CAD	9 (2529)	-0.02 (-0.14 to 0.10)	0.05	0.71
T2DM	6 (1033)	-0.07 (-0.31 to 0.17)	0.02	0.56
Hypertension	12 (1924)	-0.17 (-0.46 to 0.11)	<0.001	0.23
Healthy or control	20 (2486)	-0.07 (-0.17 to 0.04)	0.07	0.24

CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; HWE, Hardy-Weinberg equilibrium; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; NO, nitric oxide; TC, total cholesterol; T2DM, type 2 diabetes mellitus; TG, triglycerides.

Associations of the rs1799983 polymorphism with plasma lipid levels

The outcomes of the analysis on all comparisons showed that T carriers had higher levels of TC (MD 0.13 mmol/L, 95% CI 0.06 to 0.20 mmol/L, $p < 0.001$) and LDL-C (MD 0.14 mmol/L, 95% CI 0.05 to 0.22 mmol/L, $p = 0.002$), as well as lower levels of HDL-C (MD -0.07 mmol/L, 95% CI -0.14 to -0.00 mmol/L, $p = 0.04$) than the non-carriers (table 1, figures 3–5). No association was detected between the rs1799983 polymorphism and TG levels (table 1). When the analyses were limited to the studies in HWE, significant associations of the rs1799983 polymorphism with higher levels of TC (MD 0.07 mmol/L, 95% CI 0.03 to 0.11 mmol/L, $p = 0.01$) and LDL-C (MD 0.10 mmol/L, 95% CI 0.05 to 0.14 mmol/L, $p = 0.01$), and lower levels of HDL-C (MD -0.08 mmol/L, 95% CI -0.16 to -0.00 mmol/L, $p = 0.05$) were also detected.

The subgroup analyses stratified by the characteristics of the subjects were performed. The significant associations of the rs1799983 polymorphism with higher levels of TG (MD 0.16 mmol/L, 95% CI 0.04 to 0.28 mmol/L, $p = 0.01$) and LDL-C (MD 0.19 mmol/L, 95% CI 0.05 to 0.33 mmol/L, $p = 0.01$) were detected in Caucasians, but not in Asians and other ethnicities. The rs1799983 polymorphism was significantly associated with higher levels of TC in both Caucasians (MD 0.17 mmol/L, 95% CI 0.05 to 0.28 mmol/L, $p = 0.01$) and Asians (MD 0.11 mmol/L, 95% CI 0.04 to 0.18 mmol/L, $p = 0.001$). When health status was taken into account, significant associations of the rs1799983 polymorphism with higher levels of TC (MD 0.20 mmol/L, 95% CI 0.02 to 0.39 mmol/L, $p = 0.03$) were detected in patients with CAD, but not in patients with T2DM, patients with hypertension and healthy subjects.

Evaluation of heterogeneity

There was significant heterogeneity in the association analysis for NO ($I^2 = 67.5\%$, $p_{\text{heterogeneity}} < 0.01$). Three comparisons

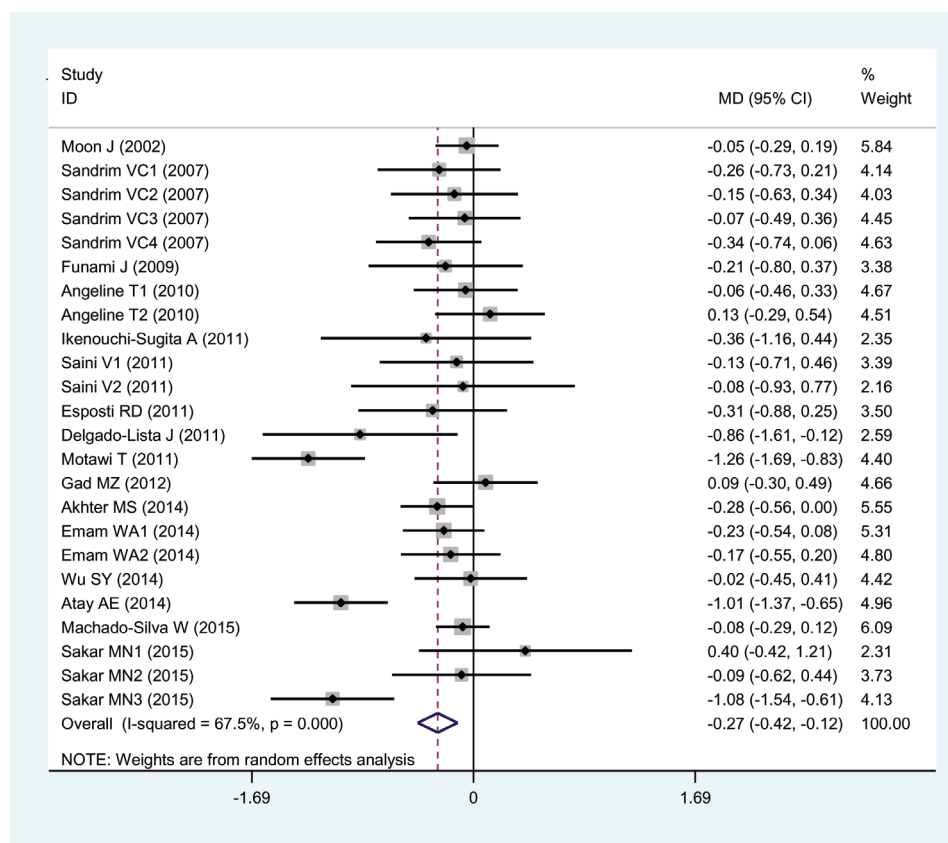


Figure 2 Forest plot of the meta-analysis between the *NOS3* rs1799983 polymorphism and circulating nitric oxide (NO) levels. MD, mean difference.

(Motawi *et al.*¹⁰ Atay *et al.*¹⁹ and Sakar *et al.*²¹) were identified as the main contributors to the heterogeneity by using Galbraith plot. The MD value and 95% CI (MD $-0.13 \mu\text{mol/L}$, 95% CI -0.22 to $-0.04 \mu\text{mol/L}$, $p_{\text{heterogeneity}} = 0.89$, $p_{\text{MD}} < 0.001$) did not change substantially after excluding these outlier comparisons (table 2).

In the association analysis for lipids, there was significant heterogeneity in the overall analysis for TG ($I^2 = 74.1\%$, $p_{\text{heterogeneity}} < 0.001$), TC ($I^2 = 64.7\%$, $p_{\text{heterogeneity}} < 0.001$), LDL-C ($I^2 = 70.2\%$, $p_{\text{heterogeneity}} < 0.001$) and HDL-C ($I^2 = 61.4\%$, $p_{\text{heterogeneity}} < 0.001$). Nine, nine, six and five comparisons were identified as the main contributors to the heterogeneity for TG, TC, LDL-C and HDL-C, respectively. The MD values and 95% CIs of TG (MD 0.00 mmol/L , 95% CI -0.04 to 0.04 mmol/L , $p_{\text{heterogeneity}} = 0.11$, $p_{\text{MD}} = 0.83$), TC (MD 0.08 mmol/L , 95% CI 0.04 to 0.12 mmol/L , $p_{\text{heterogeneity}} = 0.82$, $p_{\text{MD}} = 0.008$), LDL-C (MD 0.08 mmol/L , 95% CI 0.03 to 0.12 mmol/L , $p_{\text{heterogeneity}} = 0.29$, $p_{\text{MD}} = 0.002$) and HDL-C (MD -0.04 mmol/L , 95% CI -0.09 to -0.00 mmol/L , $p_{\text{heterogeneity}} = 0.57$, $p_{\text{MD}} = 0.05$) did not change substantially after excluding these outlier comparisons (table 2).

Publication bias test

Begg's test and Egger's test were used to evaluate the publication bias of the included studies, and no publication bias was detected.

DISCUSSION

Several meta-analyses^{77–79} demonstrated that T allele of the rs1799983 polymorphism in *NOS3* is significantly associated with increased risk of CAD, but the underlying mechanisms have not yet been clarified. In the present study, we found that T allele of the rs1799983 polymorphism is significantly associated with

decreased level of NO and abnormal levels of blood lipids. It suggests that the association between the rs1799983 polymorphism and CAD may be partly mediated by low NO level as well as dyslipidaemia caused by the variant T allele.

The underlying mechanisms whereby low level of circulating NO is associated with high risk of CAD are as follows: (1) Increase in blood pressure. NO is crucial in helping to maintain a normal blood pressure. More effective than any other factors in the body, NO can dilate the smooth muscle of blood vessels. With this dilation, the vessels can relax and allow blood to flow easily through them. As a result, the blood pressure lowers. Two studies^{80,81} demonstrated that an oral NO supplement appears to lower blood pressure in patients with hypertension and might be beneficial as a routine supplementation for cardiovascular protection. (2) Leading to endothelial dysfunction. NO plays an important role in the preservation of endothelial function.⁸² The decreased production of NO in some conditions causes serious problems in endothelial equilibrium, and which is the reason why numerous therapies have been investigated to assess the possibility of reversing endothelial dysfunction by enhancing the release of NO from the endothelium.^{83,84} (3) Leading to oxidative stress. NO acts as an important antioxidant agent against Fenton's reaction which generates highly reactive hydroxyl radicals.^{85,86} Hydroxyl radicals can lead to many disturbances which contribute to the endothelial dysfunction and act as a starting point for atherosclerosis.⁸⁷ Dyslipidaemia is closely associated with the progression of coronary atherosclerosis, and it accounts for around 50% of the population-attributable risk of CAD.⁸⁸ According to 2013 American College of Cardiology/American Heart Association blood cholesterol guidelines⁸⁹ and Adult Treatment Panel III guidelines⁹⁰ of the USA, LDL-C is considered as a major cause of CAD and used as the primary target for

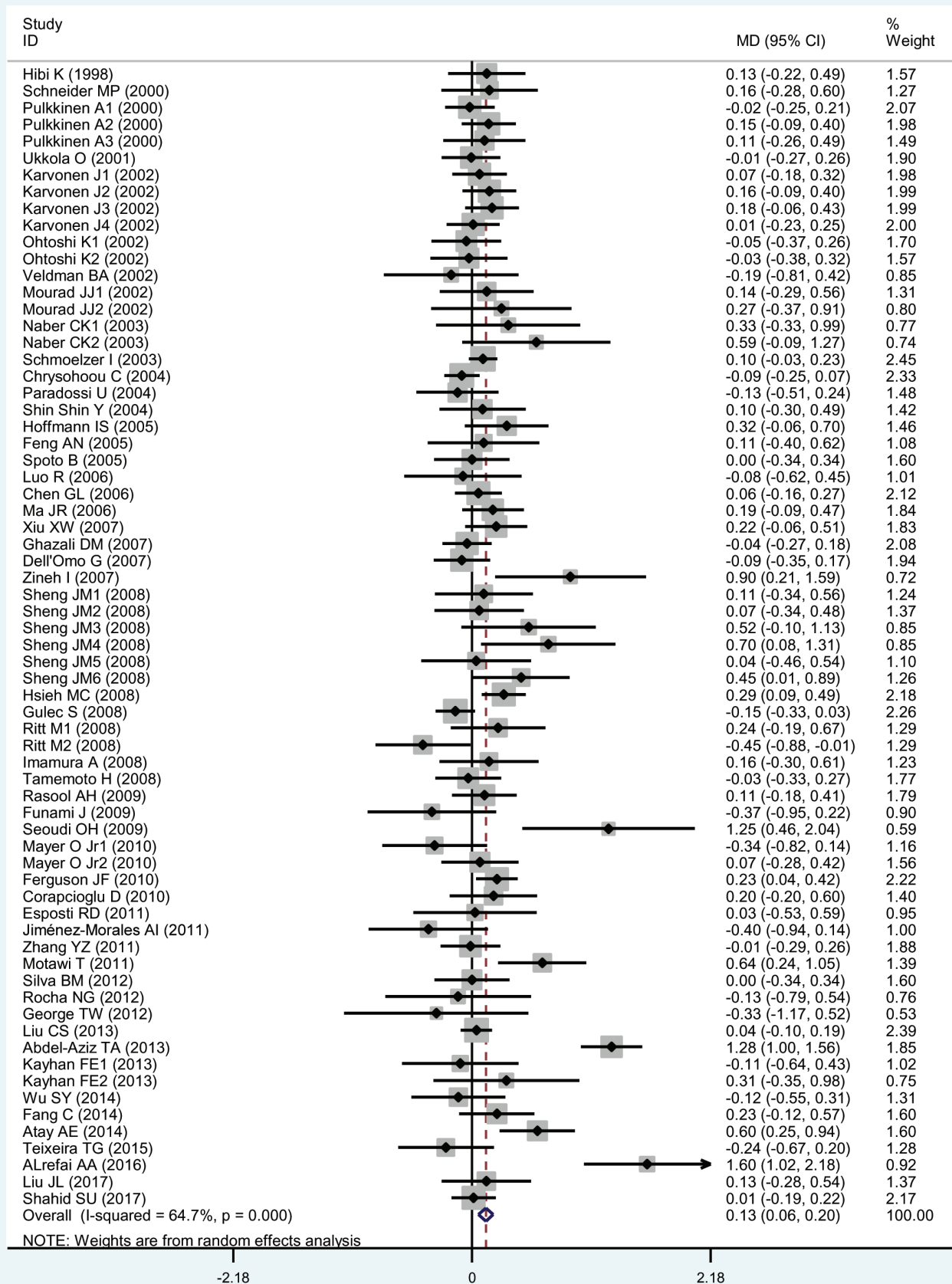


Figure 3 Forest plot of the meta-analysis between the NOS3 rs1799983 polymorphism and plasma total cholesterol (TC) levels. MD, mean difference.

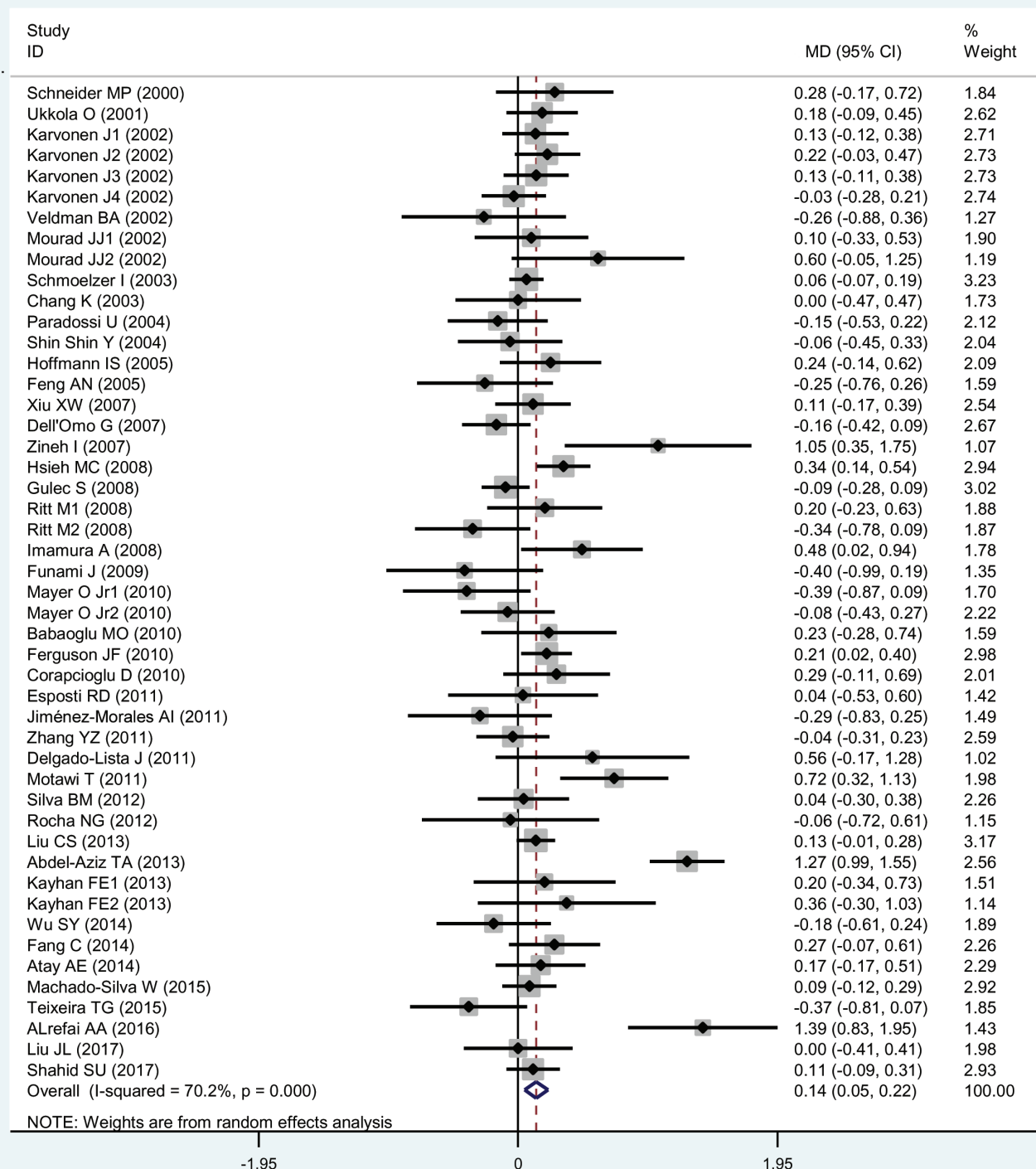


Figure 4 Forest plot of the meta-analysis between the *NOS3* rs1799983 polymorphism and plasma low-density lipoprotein cholesterol (LDL-C) levels. MD, mean difference.

therapy, and other lipid parameters are used as the secondary or supplementary targets.

Several possible reasons could be proposed to explain the association between the rs1799983 polymorphism and circulating NO level. First, the enzyme activity of *NOS3* could have been affected by the variant T allele since the nucleotide substitution (G→T) leads to the substitution of amino acid residue (Glu→Asp) in the polypeptide. Glutamic acid is a polar and

acidic amino acid which is usually located at the active site of an enzyme. Conceivably, the enzyme activity of *NOS3* can be affected if the essential glutamic acid residue is replaced by another amino acid residue. *NOS3* is responsible for synthesising circulating NO, and the change in activities will no doubt affect the production of NO. Dosenko *et al*⁹¹ measured the activities of *NOS3* enzyme by fluorimetric detection system in isolated human platelets and found that the eNOS activities

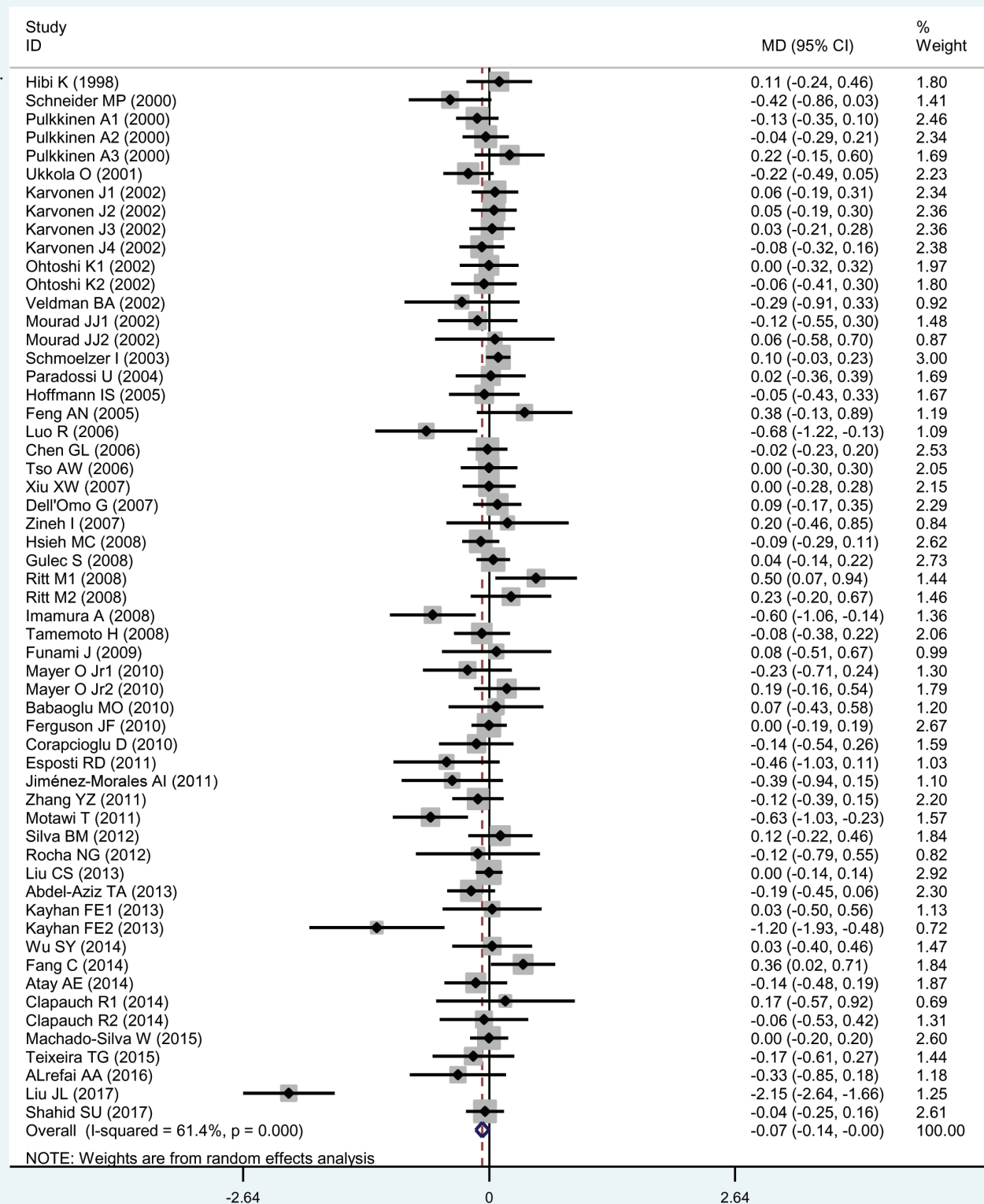


Figure 5 Forest plot of the meta-analysis between the *NOS3* rs1799983 polymorphism and plasma high-density lipoprotein cholesterol (HDL-C) levels. MD, mean difference.

accompanying the TT genotype were lower than that in wild-type GG genotype, although the difference was not statistically significant. Second, the T allele affects splicing, processing and stability of *NOS3* mRNA, resulting in reduced protein

expression. The rs1799983 polymorphism is formed by a transversion from G to T. In single-nucleotide polymorphisms, transversion (interchange between purine nucleotide and pyrimidine nucleotide) usually has more adverse effects than transition

Table 2 Meta-analysis between the *NOS3* rs1799983 polymorphism and plasma levels of NO or lipids (after excluding the studies with heterogeneity)

Groups or subgroups	Comparisons (subjects)	MD (95% CI)	P _{heterogeneity}	P _{MD}
NO				
All	21 (2381)	-0.13 (-0.22 to -0.04)	0.89	<0.001
Male	3 (240)	-0.09 (-0.36 to 0.18)	0.07	0.52
Female	4 (222)	-0.12 (-0.42 to 0.18)	0.55	0.44
Caucasian	5 (495)	-0.15 (-0.33 to 0.03)	0.23	0.10
Asian	8 (1065)	-0.11 (-0.25 to 0.03)	0.85	0.11
Other ethnic	8 (821)	-0.13 (-0.27 to 0.01)	0.81	0.06
CAD	3 (531)	-0.08 (-0.25 to 0.09)	0.98	0.35
Healthy or control	10 (1252)	-0.15 (-0.28 to -0.03)	0.40	0.02
TG				
All	54 (11 404)	0.00 (-0.04 to 0.04)	0.11	0.83
Male	5 (1125)	-0.01 (-0.13 to 0.11)	0.12	0.82
Female	6 (469)	0.04 (-0.16 to 0.23)	0.02	0.72
Caucasian	24 (5332)	0.06 (0.01 to 0.12)	0.25	0.02
Asian	16 (4040)	-0.09 (-0.16 to -0.01)	0.43	0.03
Other ethnic	14 (2032)	-0.03 (-0.12 to 0.06)	0.45	0.52
CAD	7 (2178)	-0.01 (-0.10 to 0.08)	0.92	0.83
T2DM	10 (1480)	-0.04 (-0.16 to 0.07)	0.29	0.47
Hypertension	6 (1297)	-0.10 (-0.22 to 0.01)	0.45	0.08
Healthy or control	21 (3019)	0.07 (-0.01 to 0.14)	0.20	0.08
TC				
All	59 (11 365)	0.08 (0.04 to 0.12)	0.82	0.008
Male	7 (1457)	0.08 (-0.03 to 0.18)	0.77	0.15
Female	4 (303)	-0.11 (-0.38 to 0.16)	0.45	0.41
Caucasian	27 (5074)	0.08 (0.02 to 0.13)	0.66	0.01
Asian	23 (5173)	0.11 (0.04 to 0.18)	0.61	0.001
Other ethnic	9 (1118)	0.01 (-0.12 to 0.14)	0.87	0.89
CAD	7 (1405)	0.10 (-0.01 to 0.21)	0.48	0.08
T2DM	9 (1480)	0.08 (-0.04 to 0.20)	0.94	0.19
Hypertension	10 (1824)	0.06 (-0.04 to 0.15)	0.95	0.28
Healthy or control	21 (2767)	0.09 (0.01 to 0.17)	0.64	0.03
LDL-C				
All	42 (7842)	0.08 (0.03 to 0.12)	0.29	0.002
Male	8 (1497)	0.09 (-0.01 to 0.20)	0.17	0.07
Female	5 (444)	-0.00 (-0.21 to 0.20)	0.07	0.98
Caucasian	22 (4305)	0.07 (0.01 to 0.13)	0.13	0.02
Asian	10 (2475)	0.08 (-0.01 to 0.18)	0.32	0.08
Other ethnic	10 (1062)	0.09 (-0.03 to 0.21)	0.66	0.15
CAD	3 (800)	0.14 (-0.01 to 0.29)	0.71	0.06
T2DM	4 (573)	0.16 (-0.02 to 0.33)	0.65	0.09
Hypertension	9 (1459)	0.05 (-0.06 to 0.16)	0.28	0.34
Healthy or control	18 (2114)	0.07 (-0.02 to 0.16)	0.14	0.12
HDL-C				
All	52 (10 076)	-0.04 (-0.09 to -0.00)	0.57	0.05
Male	7 (1457)	-0.01 (-0.12 to 0.09)	0.22	0.83
Female	7 (543)	-0.11 (-0.30 to 0.08)	0.83	0.26
Caucasian	26 (4359)	-0.05 (-0.11 to 0.01)	0.33	0.10
Asian	14 (4118)	-0.04 (-0.12 to 0.03)	0.27	0.24
Other ethnic	12 (1599)	-0.02 (-0.12 to 0.08)	0.92	0.72
CAD	8 (2376)	-0.04 (-0.13 to 0.04)	0.16	0.34
T2DM	5 (949)	-0.14 (-0.29 to 0.01)	0.23	0.07
Hypertension	11 (1803)	0.01 (-0.09 to 0.11)	0.77	0.84

Continued

Table 2 Continued

Groups or subgroups	Comparisons (subjects)	MD (95% CI)	P _{heterogeneity}	P _{MD}
Healthy or control	19 (2451)	-0.03 (-0.11 to 0.05)	0.40	0.49

CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; NO, nitric oxide; TC, total cholesterol; T2DM, type 2 diabetes mellitus; TG, triglycerides.

which is the interchange of purine nucleotides or pyrimidine nucleotides. In a clinical study in patients with hypertension, the mRNA levels of *NOS3* were measured in isolated platelets and the results showed that the *NOS3* mRNA levels accompanying the TT genotype are lower than those found in wild-type GG genotype.⁹¹ However, Doshi *et al*⁹² concluded that the rs1799983 polymorphism was not associated with either *NOS3* mRNA or protein in explanted human hearts. Further studies will be needed to investigate the relationship between the rs1799983 polymorphism and the expression of *NOS3*.

In this meta-analysis, significantly higher levels of TC and LDL-C as well as lower levels of HDL-C were detected in T carriers comparing with the subjects with the GG genotype. The mechanisms in which the rs1799983 polymorphism modulates plasma lipid levels have not been clarified yet. One explanation could be that the rs1799983 polymorphism indirectly affects plasma lipid levels through the mediation of NO. As mentioned above, reduced NO levels in blood can cause increase in blood pressure, endothelial dysfunction and oxidative stress. All these events are likely to trigger the development of dyslipidaemia.^{93–95} Yatera *et al*⁹⁶ developed the mice lacking all three NOS (*NOS1*, *NOS2* and *NOS3*) and these mice experienced severe dyslipidaemia, atherosclerosis and sudden cardiac death in response to a high-fat diet for 3–5 months.

The dominant model (GG vs TT +GT) was adopted in this meta-analysis since most of the included studies used this model. In the subgroup analysis stratified by ethnicities, the differences between the genotypes were mainly from Caucasians, whose MD values were larger than those calculated in Asians and the subjects of other ethnicities (table 1). It indicates that there is an interaction between the rs1799983 polymorphism and ethnicity in modulating plasma levels of NO and lipids. There was significant heterogeneity in the total analysis for NO, TG, TC, LDL-C and HDL-C. Outlier studies were identified for each variable by Galbraith plots. Reanalysis was done for each variable after excluding the outlier studies, but no significant changes in MD values and 95% CIs were found (table 2). It indicates that the associations between the rs1799983 polymorphism and the levels of NO and lipids are very robust.

There were several limitations in the present meta-analysis. First, a large number of genes and environmental factors (eg, diet, exercise and smoking status) are associated with plasma lipid levels, but the interactions between the rs1799983 polymorphism and other genes or environmental factors on plasma lipid levels were not explored in this meta-analysis as few original data were available in the included studies. Second, a relatively small number of subjects were included in the association analysis between the rs1799983 polymorphism and NO due to a limited number of studies which met the inclusion criteria. It reduces the statistic power and may cause type I error. Third, only the studies which were published in English or Chinese were included in this meta-analysis as it is very difficult to get the full articles published in various languages.

Main messages

- The *NOS3* rs1799983 polymorphism is significantly associated with circulating levels of nitric oxide (NO) and lipids.
- The association between the *NOS3* rs1799983 polymorphism and coronary artery disease may be partly mediated by abnormal NO and lipid levels.

Current research questions

- Is it possible that endothelial nitric oxide synthase (NOS3) acts as a new target for the treatment of coronary artery disease?
- What are the underlying mechanisms whereby the *NOS3* rs1799983 polymorphism affects plasma lipid levels?
- How does the *NOS3* rs1799983 polymorphism modulate plasma levels of nitric oxide?

CONCLUSION

The meta-analysis suggests that the rs1799983 polymorphism is significantly associated with abnormal levels of NO and lipids, which may partly explain the significant association between the rs1799983 polymorphism and CAD.

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Contributors YS and ZhiL conceived the study, participated in the design and drafted the manuscript. ZhiL, AJ, ZhanL, IM and AA carried out the study searches and collected the data. YS, ZhiL and ZhanL performed the statistical analyses.

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