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Review Article

Association between PON1 L55M polymorphisms and risk of coronary heart disease: a meta-analysis based on 46 case-control studies

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Abstract: The purpose of the current meta-analysis was to explore the association between PON1 L55M polymorphisms and risk of CHD. Relevant studies were enrolled after a systematical literature search of Pubmed, Embase, OVID, and Web of Science databases in English. Odds ratios (ORs) with 95% confidence intervals (CIs) were used in different genetic models to evaluate the strength of association. Funnel plots and Egger's tests were performed to evaluate publication bias. Subgroup analyses were conducted by ethnicity, diagnosis, sample size, and results of HWE testing. A total of 46 studies, involving 15,554 cases and 18,137 controls, were included in this meta-analysis. Overall analysis showed an insignificant association between PON1 L55M polymorphisms and CHD under allelic (OR: 1.06, 95% CI: 0.99-1.13, P = 0.118), homozygous (OR: 1.12, 95% CI: 0.95-1.31, P = 0.166), heterozygous (OR: 1.11, 95% CI: 0.95-1.31, P = 0.199), recessive (OR: 1.04, 95% CI: 0.97-1.11, P = 0.34), and dominant (OR: 1.13, 95% CI: 0.96-1.33, P = 0.13) models. However, subgroup analyses showed a significant association in Asians. No association was observed between PON1 L55M polymorphisms and MI. Subgroup analyses of studies with sample sizes > 500 and p of HWE testing > 0.05 yielded insignificant results. In conclusion, L55M polymorphisms in PON1 genes are not associated with susceptibility to CHD. However, the association was significant in Asian populations. More high-quality studies should be carried out to validate present conclusions.

Keywords: Coronary heart disease, PON1, gene, L55M, polymorphism

Introduction

Epidemiological studies have shown that coronary heart disease (CHD) is one of the major causes of high morbidity and mortality, worldwide [1, 2]. To date, a decrease of plasma high-density lipoprotein cholesterol (HDL-C) is one of the strongest risk factors for CHD. The antioxidant activity of HDL is mainly due to the paraoxonase (PON) enzyme, which can prevent the formation of oxidized LDL (ox-LDL) and to inactivate LDL-derived oxidized phospholipids [3, 4]. Genetic polymorphisms in the PON gene might affect the concentration and activity of PON enzymes, thus impacting anti-LDL oxidant functions of HDL [5]. The human paraoxonase 1 (PON1) gene is located on the long arm of chromosome 7 (7q 21.3-22.1) [6, 7]. PON1 is a 43 KDa calcium-dependent antioxidant glycoprotein. It is synthesized in the liver and secreted into the circulation. As an important component of HDL, it protects HDL from oxidation and maintains the anti-atherosclerosis function of HDL. Polymorphisms of PON1 genes are known to affect PON1 activity, thereby increasing CHD risk. There are two main polymorphisms in the coding region of PON1, L55M (163T > A) and Q192R (575A > G). At codon 55, leucine (L) is replaced by methionine (M). At codon 192, glutamine (Q) is replaced by arginine (R). It has been shown that the L55M variant modulates PON1 concentrations and levels and the Q192R variant modulates enzymatic activity [8, 9]. Numerous case-control studies have been conducted to explore the association between these two polymorphisms and risk of coronary heart disease (CHD). Several meta-analyses have been conducted. Most have found a sig-

nificant relationship between Q192R polymorphisms and CHD [10-12]. However, there have been contradictory results concerning the association between L55M polymorphisms and CHD. Of the three meta-analyses on the L55M variant published at present, one [11] showed a significant association with CHD in certain populations. However, the other two [10, 13] did not. Furthermore, in recent years, several new studies have been published, showing both significant results [14, 15] and insignificant results [16, 17]. Aiming to draw updated and consolidated conclusions concerning the association between L55M and susceptibility to CHD, the current meta-analysis was conducted.

Material and methods

The current meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [18]. Ethical approval and patient content were not necessary, as this meta-analysis was based on previously published studies.

A systematic computerized literature search was performed identifying relevant articles in PubMed, Embase, OVID, and Web of Science databases up to September 10, 2018. The following search terms were used: ("paraoxonase 1" or "PON1") and ("L55M" or "rs854560") and ("polymorphism") and ("coronary heart disease" or "coronary artery disease" or "coronary diseases"). A manual search was also conducted. It was based on references of relevant review articles of all identified individual studies, aiming to discover more eligible studies.

Inclusion criteria: (1) Case-control studies investigating the association between L55M polymorphism and CHD; (2) Provided ample data on allele or genotype distribution in patients and controls; and (3) Studies written in English. Exclusion criteria: (1) Duplicated data; (2) Studies that provided limited data for extraction; and (3) Abstract-only articles, reviews, letters, meta-analyses, or unpublished studies.

Two authors (J-YZ, MD, and Y-FJ, MD), independently, read the 32 included studies, extracting useful data from each. Conflicts were discussed with a third investigator (Y-FZ, PhD). The following data were extracted: Author, year of publication, geographical location, ethnicity, total number of cases and controls, source of controls, genotyping method, and genotype distribution. Study quality was evaluated based on the 9-point Newcastle-Ottawa Scale (NOS) [19].

For each included study, Hardy-Weinberg equilibrium (HWE) testing was conducted to access genotype frequencies of the polymorphisms of included populations. This study investigated the strength of association between PON1 L55M polymorphisms and susceptibility to CHD by combining odds ratio (ORs) and 95% confidence intervals (CIs) under a fixed or random-effects model, according to heterogeneity calculated with the I^2 test. When I^2 > 50% (indicating significant heterogeneity), a random-effects model (Der Simonian and Laird method) was adopted. Otherwise, a fixed-effects model (Mantel-Haenszel method) was used. Subgroup analyses were performed to identify possible underlying heterogeneity, according to ethnicity, diagnostic (whether it was MI or not), sample size, and results of HWE testing. Overall and subgroup analyses were conducted using five genetic models, including the allele model (L vs. M), homozygote model (LL vs. MM), heterozygote model (LM vs. MM), recessive model (LL vs. LM+MM), and dominant model (LL+LM vs. MM). Sensitive analysis was performed by pooling ORs repeated with omission of each study, evaluating the influence of single studies on the overall estimate. Finally, the current study investigated publication bias via constructing funnel plots and performing Egger's tests. Significant publication bias is indicated when P<0.05. This meta-analysis was performed using Stata version 12.0 (Stata corporation).

Results

The literature search identified a total of 845 records. After removing duplicate studies, 423 studies remained for screening. Of these, 352 were excluded. A total of 71 studies were read via full-texts. Of these, another 25 articles were excluded because of unmatched study designs (n = 11), insufficient data (n = 8), and not relevant to CHD (n = 6). The complete procedure regarding literature selection and exclusion is shown in **Figure 1**. Eventually, 46 studies [14-17, 20-61], including 15,554 cases and 18,137

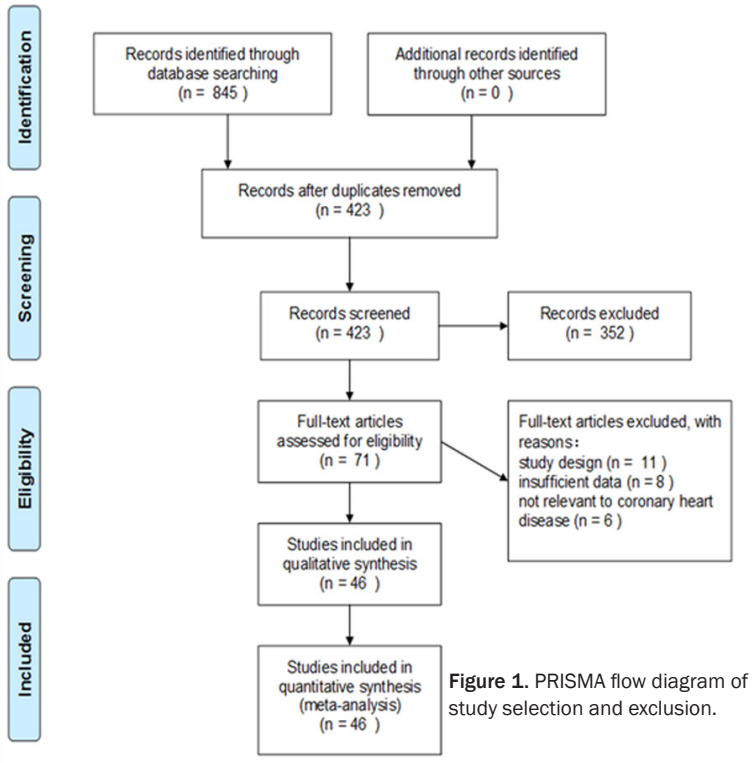


Figure 1. PRISMA flow diagram of study selection and exclusion.

controls, were eligible for this meta-analysis examining the relationship between PON1 gene L55M polymorphisms and CHD. Characteristics of included studies are shown in **Table 1**. Sample sizes ranged from 45 to 3,114 for all eligible articles. Ethnicities of included studies were Asians (n = 7) and Caucasians (n = 39). A total of 13 studies specifically explored the association between L55M polymorphisms and myocardial infarction (MI), while the other 31 studies did not restrict the case-population to patients with MI. Fourteen studies did not fit in with HWE testing. Results of the NOS are shown in **Table 2**. Genotype distributions and allele frequencies in cases and controls of each study are shown in **Table 3**.

Pooling data of all included studies, results indicated an insignificant association between PON1 gene L55M polymorphisms and CHD under allelic (OR: 1.06, 95% CI: 0.99-1.13, P = 0.118, I^2 = 65%), homozygous (OR: 1.12, 95% CI: 0.95-1.31, P = 0.166, I^2 = 64%), heterozygous (OR: 1.11, 95% CI: 0.95-1.31, P = 0.199, I^2 = 68%), recessive (OR: 1.04, 95% CI: 0.97-1.11, P = 0.34, I^2 = 38%), and dominant models (OR: 1.13, 95% CI: 0.96-1.33, P = 0.13, I^2 = 71%) (**Figure 2**).

However, according to subgroup analyses by ethnicity, a higher risk was detected in Asians under four models, including allelic (OR: 1.18, 95% CI: 1.01-1.34, P = 0.035, I^2 = 0%), homozygous (OR: 1.80, 95% CI: 1.11-2.92, P = 0.017, I^2 = 0%), heterozygous (OR: 2.14, 95% CI: 1.38-3.33, P = 0.001, I^2 = 0%), and dominant genetic models (OR: 2.08, 95% CI: 1.38-3.12, P<0.001, I^2 = 0%) (**Figure 3**). However, in the Caucasian subgroup, the association remained insignificant. According to subgroup analyses stratified by source of control and diagnostics, no association among L55M polymorphisms and CHD was observed in any of the models. The relationship seemed weaker in patients with myocardial infarction (allelic model: OR 1.006, 95% CI, 0.94-1.08, P = 0.849, I^2 = 0%). Subgroup analyses was also conducted by sample size and results of HWE testing. Significant results were found among small studies (n<500) and relatively low-quality studies (HWE test: $P \leq 0.05$). In studies with a sample of $n \geq 500$ and *p* value of HWE testing > 0.05, the relationship between L55M polymorphisms and CHD remained insignificant. Results of subgroup analyses are shown in **Table 4**. Forest plots and funnel plots of subgroup analyses are shown in Supplementary Figures 1, 2, 3, 4, 5.

Sensitivity analyses was performed to check whether the exclusion of each study would alter pooled ORs. Results were not altered after the omission of any individual study, suggesting that outcomes were statistically robust (**Figure 4**).

Begg's funnel plots and Egger's tests were performed to evaluate publication bias of included studies. As shown in the funnel plot (**Figure 2F**), the 46 studies were symmetrically distributed on the two sides, suggesting no publication bias (Egger's test: P = 0.051). Funnel plot results and insignificant *P*-values of Egger's testing (P = 0.051) suggest that publication bias existed, to some extent, possibly due to

Table 1. Characteristics of studies included for this meta-analysis

Author	Year	Country	Ethnicity	Age, y		Sex (M/F)		MI	Source of controls	Genotyping method	NOS score	HWE test
				Case	Control	Case	Control					
Zama et al. [20]	1997	Japan	Asian	62.6 (9.7)	48.3 (6.3)	35/30	54/40	No	PB	PCR-RFLP	7	0.59
Sanghera et al. [21]	1998	Singapore	Asian	54.8 (0.9)	43.3 (1.0)	218/15	338/26	No	PB	PCR-RFLP	7	0.02
Cascorbi et al. [24]	1999	Germany	Caucasian	60.6 (5.5)	60.5 (6)	759/241	759/241	No	HB	Direct sequencing	7	0.58
Hasselwander et al. [23]	1999	Ireland	Caucasian	55.8 (10.3)	43.1 (16.0)	70/33	234/154	No	HB	PCR-RFLP	7	0.24
Ayub et al. [22]	1999	UK	Caucasian	55.7 (7.8)	49 (8.0)	38/12	37/11	Yes	HB	PCR-RFLP	7	0.36
Imai et al. [27]	2000	Japan	Asian	62.5 (9.1)	63.6 (9.1)	184/26	321/110	No	HB	PCR-RFLP	7	0.08
Senbanergee et al. [25]	2000	Mexico	Caucasian	57 (0.7)	57 (0.7)	243/24	261/27	Yes	PB	Direct sequencing	6	0.15
Gardemann et al. [26]	2000	Germany	Caucasian	62.7 (9.3)	55.3 (10.2)	535/0	1742/0	Partly	PB	PCR- RFLP	8	0.97
Mackness et al. [28]	2001	UK	Caucasian	58.5 (10.2)	42.2 (12.2)	302/115	147/135	No	PB	PCR-RFLP	8	0.01
Arca et al. [33]	2002	Italy	Caucasian	60.5 (8.7)	59.4 (9.1)	323/72	90/108	Partly	PB	PCR	7	0.93
Ferre et al. [30]	2002	Spain	Caucasian	60.6 (11.8)	62.1 (16.4)	215/0	215/0	Yes	PB	Restriction isotyping	8	0.11
Watzinger et al. [31]	2002	Austria	Caucasian	59.6 (5.9)	59.8 (6.5)	23/20	130/130	No	PB	PCR-RFLP	9	0.42
Letellier et al. [32]	2002	France	Caucasian	60 (9.6)	46.7 (10.9)	51/20	52/53	No	PB	PCR-RFLP	7	0.057
Yamada et al. [29]	2002	Japan	Asian	-	-	219/226	232/232	Yes	HB	PCR-RFLP	7	0.24
Robertson et al. [34]	2003	UK	Caucasian	56.6 (3.6)	56 (3.4)	192/0	2510/0	No	PB	PCR-RFLP	7	0.90
Martinelli et al. [35]	2004	Italy	Caucasian	60.7 (9.5)	58 (12.3)	502/116	186/86	No	HB	PCR	7	0.53
Oliveira et al. [36]	2004	Brazil	Caucasian	54.3 (12.2)	51.6 (13.2)	230/122	246/134	No	HB	PCR-RFLP	7	0.35
Tobin et al. [37]	2004	UK	Caucasian	61.9 (9.2)	58.6 (10.7)	372/175	313/192	Yes	PB	PCR-RFLP	7	0.90
Martinelli et al. [38]	2005	Italy	Caucasian	60.6 (9.4)	57.8 (12.3)	520/122	187/86	No	HB	PCR-RFLP	8	0.85
Kerkeni et al. [39]	2006	Tunisia	Caucasian	59 (10)	54 (10)	74/26	87/33	No	PB	PCR-RFLP	7	0.04
Blatter et al. [40]	2006	Switzerland	Caucasian	60.5 (9.6)	56.7 (10.7)	564/146	100/99	No	HB	PCR	7	0.52
Rios et al. [41]	2007	Brazil	Caucasian	55.5 (7.0)	52.3 (8.2)	196/100	65/76	No	HB	PCR-RFLP	7	<0.001
Himbergen et al. [42]	2007	Nethelands	Caucasian	61 (6)	57 (6)	0/211	0/1527	Partly	HB	PCR-RFLP	8	0.84
Saeed et al. [43]	2007	Pakistan	Caucasian	54.1 (10.7)	49.7 (11.0)	153/58	258/112	Yes	PB	PCR-RFLP	7	0.08
Ozkok et al. [45]	2008	Turkey	Caucasian	54.5 (11.3)	55.1 (4.0)	110/29	86/33	No	PB	PCR-RFLP	7	<0.001
Troughton et al. [44]	2008	UK	Caucasian	-	-	247/0	433/0	No	PB	PCR-RFLP	8	0.46
Agrawal et al. [47]	2009	India	Asian	47.5 (11.9)	44.6 (13.4)	244/41	163/37	No	PB	AS-PCR	6	0.21
Aydin et al. [53]	2009	Turkey	Caucasian	60.7 (11.0)	59.2 (10.8)	197/71	172/63	No	PB	PCR	6	<0.001
Birjmohun et al. [52]	2009	Netherlands	Caucasian	65 (8.0)	65 (8.0)	754/384	1469/768	No	PB	Direct sequencing	8	0.59
Kaman et al. [46]	2009	Turkey	Caucasian	68.1 (10.6)	56.3 (6.8)	188/89	54/38	No	HB	PCR-RFLP	7	0.62
Mukamal et al. [51]	2009	USA	Caucasian	65 (8.0)	65 (8.0)	263/243	528/490	Yes	PB	Taqman assay	7	0.40
Sesal et al. [49]	2009	Turkey	Caucasian	69.2 (9.1)	68.4 (8.7)	11/8	15/11	No	PB	PCR-RFLP	8	0.70
Taskiran et al. [50]	2009	Turkey	Caucasian	48.2 (4.3)	46.8 (5.2)	92/28	80/22	No	PB	PCR-RFLP	7	0.49
Koubaa et al. [48]	2009	Tunisia	Caucasian	61.47 (12.1)	61.2 (9.6)	64/27	59/59	No	PB	Multiplex PCR	6	0.32
Kallel et al. [54]	2010	Tunisi	Caucasian	53.9 (8.6)	50.9 (9.5)	310/0	375/0	Yes	HB	PCR-RFLP	6	0.74
Lakshmy et al. [55]	2010	India	Caucasian	52.2 (11.5)	52.0 (11.3)	108/16	169/25	Yes	HB	PCR-RFLP	7	0.02
Gupta et al. [56]	2011	India	Asian	55.9 (9.7)	43.1 (10.7)	286/64	151/149	No	PB	PCR-RFLP	7	0.08

Ahmad et al. [57]	2012	India	Asian	55.6 (8.6)	45.9 (10.4)	175/29	113/65	No	HB	PCR-RFLP	7	0.58
Rejeb et al. [58]	2013	Tunisia	Caucasian	60.6 (10.6)	59.4 (11.9)	140/72	58/46	No	HB	PCR-RFLP	7	0.05
Grubisa et al. [59]	2013	Serbia	Caucasian	65.5 (10.4)	63.2 (9.9)	37/23	62/38	No	PB	PCR-RFLP	8	0.57
Kang et al. [60]	2013	China	Asian	67.4 (7.4)	53.5 (8.5)	449/66	375/161	No	HB	PCR-RFLP	6	<0.001
Liu et al. [61]	2014	China	Asian	54.5 (10.3)	53.1 (10.4)	818/374	845/419	No	HB	Direct sequencing	7	0.04
Bounafaa et al. [14]	2015	Morroco	Caucasian	55 (0.6)	57.5 (0.6)	125/80	52/48	No	HB	PCR	7	0.61
Fridman et al. [15]	2016	Argentina	Caucasian	63.4 (1.5)	60 (1.3)	83/43	105/98	No	HB	PCR-RFLP	7	0.02
Kocakap et al. [16]	2016	Turkey	Caucasian	57.6 (12.3)	54.1 (12.3)	27/13	33/18	No	HB	PCR-RFLP	8	0.50
Chen et al. [17]	2017	China	Asian	55.1 (5.38)	49.0 (3.6)	42/41	37/42	No	HB	PCR-RFLP	7	0.02

control design was used in all included studies. MI = myocardial infarction. HB = hospital-based. PB = population based. NOS = Newcastle-Ottawa scale. HWE = Harty-Weinberg equilibrium. PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism. year = publication year.

Table 2. Results of Newcastle-Ottawa scale

	Selection	Comparability	Exposure
Zama et al. [20]	★★★	★★	★★
Sanghera et al. [21]	★★★★	★	★★
Cascorbi et al. [24]	★★★	★★	★★
Hasselwander et al. [23]	★★★	★★	★★
Ayub et al. [22]	★★★	★★	★★
Imai et al. [27]	★★★	★★	★★
Senbanergee et al. [25]	★★★	★	★★
Gardemann et al. [26]	★★★★	★★	★★
Mackness et al. [28]	★★★★	★★	★★
Arca et al. [33]	★★★	★★	★★
Ferr et al. [30]	★★★★	★★	★★
Watzinger et al. [31]	★★★★	★★	★★★
Letellier et al. [32]	★★★	★★	★★
Yamada [29]	★★★	★★	★★
Robertson et al. [34]	★★★	★★	★★
Martinelli et al. [35]	★★★	★★	★★
Oliveira et al. [36]	★★★	★★	★★
Tobin et al. [37]	★★★★	★★	★★
Martinelli et al. [38]	★★★	★★	★★
Kerkeni et al. [39]	★★★	★★	★★
Blatter et al. [40]	★★★	★★	★★
Rios et al. [41]	★★★	★★	★★
Himbergen et al. [42]	★★★	★★	★★★
Saeed et al. [43]	★★★★	★	★★
Ozkok et al. [45]	★★★	★★	★★★
Troughton et al. [44]	★★★★	★★	★★
Agrawal et al. [47]	★★★	★	★★
Aydin et al. [53]	★★★	★	★★
Birjmohun et al. [52]	★★★★	★★	★★
Kaman et al. [46]	★★★	★★	★★
Mukamal et al. [51]	★★★	★★	★★★
Sesal et al. [49]	★★★★	★★	★★
Taskiran et al. [50]	★★★	★	★★
Koubaa et al. [48]	★★★	★	★★
Kallel et al. [54]	★★★	★	★★
Lakshmy et al. [55]	★★★	★★	★★
Gupta et al. [56]	★★★★	★	★★
Ahmad et al. [57]	★★★	★	★★★
Rejeb et al. [58]	★★★	★★	★★
Grubisa et al. [59]	★★★★	★★	★★
Kang et al. [60]	★★★	★	★★
Liu et al. [61]	★★★	★★	★★
Bounafaa et al. [14]	★★★	★★	★★
Fridman et al. [15]	★★★	★★	★★
Kocakap et al. [16]	★★★	★★	★★★
Chen et al. [17]	★★★	★★	★★

the preferential publication of positive results from small and low-quality studies. This study used the Duval and Tweedie nonparametric "trim and fill" method to adjust for publication bias. Meta-analysis results after the "trim and fill" method drew similar conclusions (Supplementary Figure 6), indicating that present results are statistically robust.

Discussion

Lipid peroxidation has been intimately associated with the pathogenesis of atherosclerosis and arterial thrombosis, ultimately leading to coronary heart disease. On the other hand, high-density lipoproteins (HDL) have an important position in protecting against CHD, mainly relying on antioxidant components. PON1 is the main antioxidant enzyme connected with HDL particles [47, 62]. Genes encoding PON1, along with their relationship with CHD, have been extensively researched. L55M is one of the several polymorphisms that have drawn the most attention from researchers. Knowing the roles of L55M variation in occurrence and development of CHD is important for better personalized management.

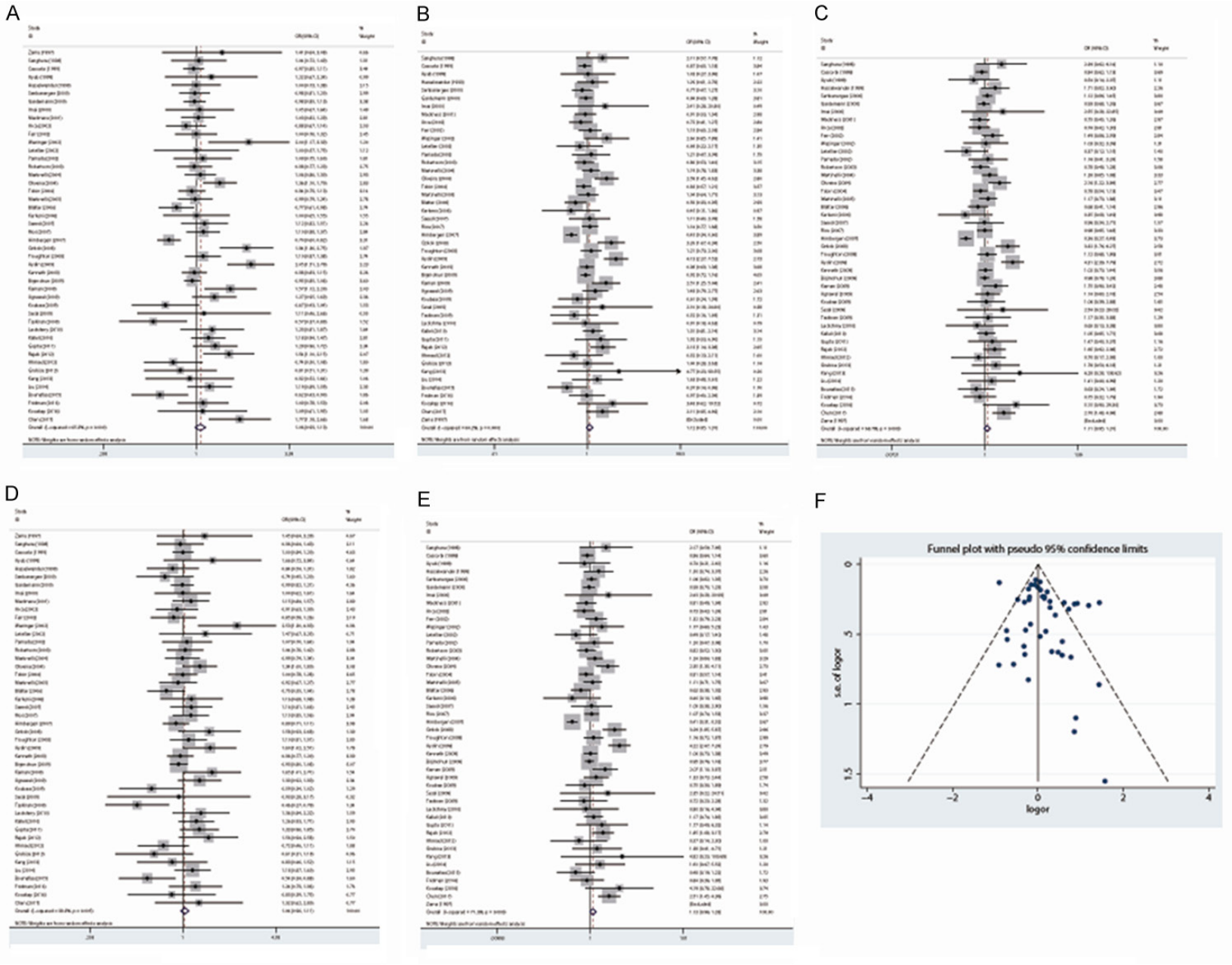
To date, many case-control studies investigating the relationship between PON1 L55M polymorphisms and risk of CHD have been published, with conflicting results. A previous meta-analysis by Hernandez-Diaz, Y et al. [11], published in 2016, included 29 relevant studies. They concluded that Pon1 L55M polymorphisms are not associated with heart disease (including CHD, CAD, and MI) in the overall population. This conclusion was not rigorous enough. The present study found that this previous meta-analysis left out some studies that could be included [21, 24, 26, 31, 32, 38, 42, 49, 50, 54, 57-61]. Furthermore, three more relevant articles [15-17] were published after the publication of that meta-analysis. Of these additional studies, 5 [15, 31, 42, 50, 58] reported an association between PON1 L55M polymorphisms and risk of CHD, while the other 12 studies [16, 17, 21, 24, 26, 32, 38, 49, 54,

Table 3. PON1 L55M polymorphism genotype distribution and allele frequency in cases and controls

Author	Genotype (N)								Allele frequency (N, %)					
	Cases				Controls				Cases			Controls		
	Total	LL	LM	MM	Total	LL	LM	MM	L	M	RAF	L	M	RAF
Zama et al. [20]	75	65	10	0	115	94	21	0	140	10	0.93	209	21	0.91
Sanghera et al. [21]	233	182	48	3	364	287	67	10	412	54	0.88	641	87	0.88
Cascorbi et al. [24]	963	433	416	114	971	436	435	100	1282	644	0.67	1307	635	0.67
Hasselwander et al. [23]	103	40	53	10	388	167	167	54	132	72	0.65	504	272	0.65
Ayub et al. [22]	50	21	17	6	48	17	26	5	59	29	0.67	60	36	0.63
Imai et al. [27]	208	179	28	1	431	371	55	5	386	30	0.93	797	65	0.92
Senbanergee et al. [25]	492	30	195	267	518	42	188	288	255	729	0.26	272	764	0.26
Gardemann et al. [26]	1742	720	787	235	535	222	245	68	2227	1257	0.64	689	381	0.64
Mackness et al. [28]	417	169	200	48	282	105	150	27	538	296	0.65	360	204	0.64
Arca et al. [35]	387	156	171	60	178	76	81	21	483	291	0.62	233	123	0.65
Ferr et al. [30]	215	78	107	30	215	86	91	38	263	167	0.61	263	167	0.61
Wazinger et al. [31]	43	27	12	4	260	104	116	40	66	20	0.77	324	196	0.62
Letellier et al. [32]	36	15	14	7	95	31	54	10	44	28	0.61	116	74	0.61
Yamada [29]	445	400	37	8	464	414	40	10	837	53	0.94	868	60	0.94
Robertson et al. [34]	172	78	71	23	2211	980	982	249	227	117	0.66	2942	1480	0.67
Martinelli et al. [35]	618	224	305	89	272	99	126	47	754	483	0.61	324	220	0.60
Oliveira et al. [36]	351	165	167	19	376	151	183	45	497	205	0.70	494	258	0.66
Tobin et al. [37]	547	221	240	86	505	204	235	66	682	412	0.62	643	367	0.64
Martinelli et al. [38]	161	227	58	446	93	117	35	245	549	343	0.62	303	187	0.62
Kerkeni et al. [39]	100	57	37	6	120	64	53	3	151	49	0.76	181	59	0.75
Blatter et al. [40]	710	249	348	113	199	85	95	21	846	574	0.60	261	137	0.66
Rios et al. [41]	444	200	145	99	269	112	94	63	545	343	0.61	318	220	0.59
Himbergen et al. [42]	422	156	146	120	601	703	210	1514	458	386	0.54	1905	1123	0.63
Saeed et al. [43]	201	127	68	6	350	209	130	11	322	80	0.80	548	162	0.78
Ozkok et al. [45]	139	51	65	23	119	32	40	47	167	111	0.60	104	134	0.44
Troughton et al. [44]	247	111	107	29	433	184	191	58	329	165	0.67	559	307	0.65
Agrawal et al. [47]	279	158	96	25	190	94	74	22	412	146	0.74	262	118	0.69
Aydin et al. [53]	221	92	103	26	136	42	45	49	267	155	0.65	129	143	0.47
Birjmohun et al. [52]	1050	424	486	140	2064	869	932	263	1334	766	0.64	2670	1458	0.65
Kaman et al. [46]	277	123	123	31	92	30	43	19	369	185	0.66	103	81	0.56
Mukamal et al. [51]	482	198	220	64	971	409	433	129	616	348	0.64	1251	691	0.64
Sesal et al. [49]	19	7	11	1	26	10	13	3	25	13	0.66	33	19	0.64
Taskiran et al. [50]	120	56	56	8	67	30	5	102	168	72	0.7	164	40	0.80
Koubaa et al. [48]	91	46	35	10	118	75	33	10	127	55	0.70	183	53	0.78
Kallel et al. [54]	310	139	135	36	375	147	178	50	413	207	0.66	472	278	0.63
Lakshmy et al. [55]	124	80	41	3	154	88	63	3	201	47	0.81	239	69	0.78
Gupta et al. [56]	350	247	99	4	300	193	101	6	593	108	0.85	487	113	0.81
Ahmad et al. [57]	204	132	66	6	178	128	47	3	330	78	0.81	303	53	0.85
Rejeb et al. [58]	212	82	89	41	104	30	42	32	253	171	0.59	102	106	0.49
Grubisa et al. [59]	60	20	36	4	100	45	46	9	76	44	0.63	136	64	0.68
Kang et al. [60]	515	491	24	0	536	515	19	2	1006	24	0.98	1049	23	0.98
Liu et al. [61]	792	709	79	4	864	795	98	7	1497	87	0.95	1616	112	0.94
Bounafaa et al. [14]	100	52	42	6	205	76	105	24	146	54	0.73	253	153	0.63
Fridman et al. [15]	203	88	98	17	126	48	69	9	274	132	0.67	165	87	0.65
Kocakap et al. [16]	69	32	35	2	45	23	17	5	99	39	0.72	63	27	0.70
Chen et al. [17]	165	29	81	55	79	11	24	44	139	191	0.42	46	112	0.29

Case-control design was used in all the included studies. RAF = risk allele frequency. Risk allele = L allele.

57, 59-61] reported no significant association. The synthesis of these results may have changed the original meta-results. Therefore, it was necessary to conduct a new meta-analysis on the association between PON1 L55M polymorphisms and risk of CHD.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Funnel plot with pseudo 95% confidence limits
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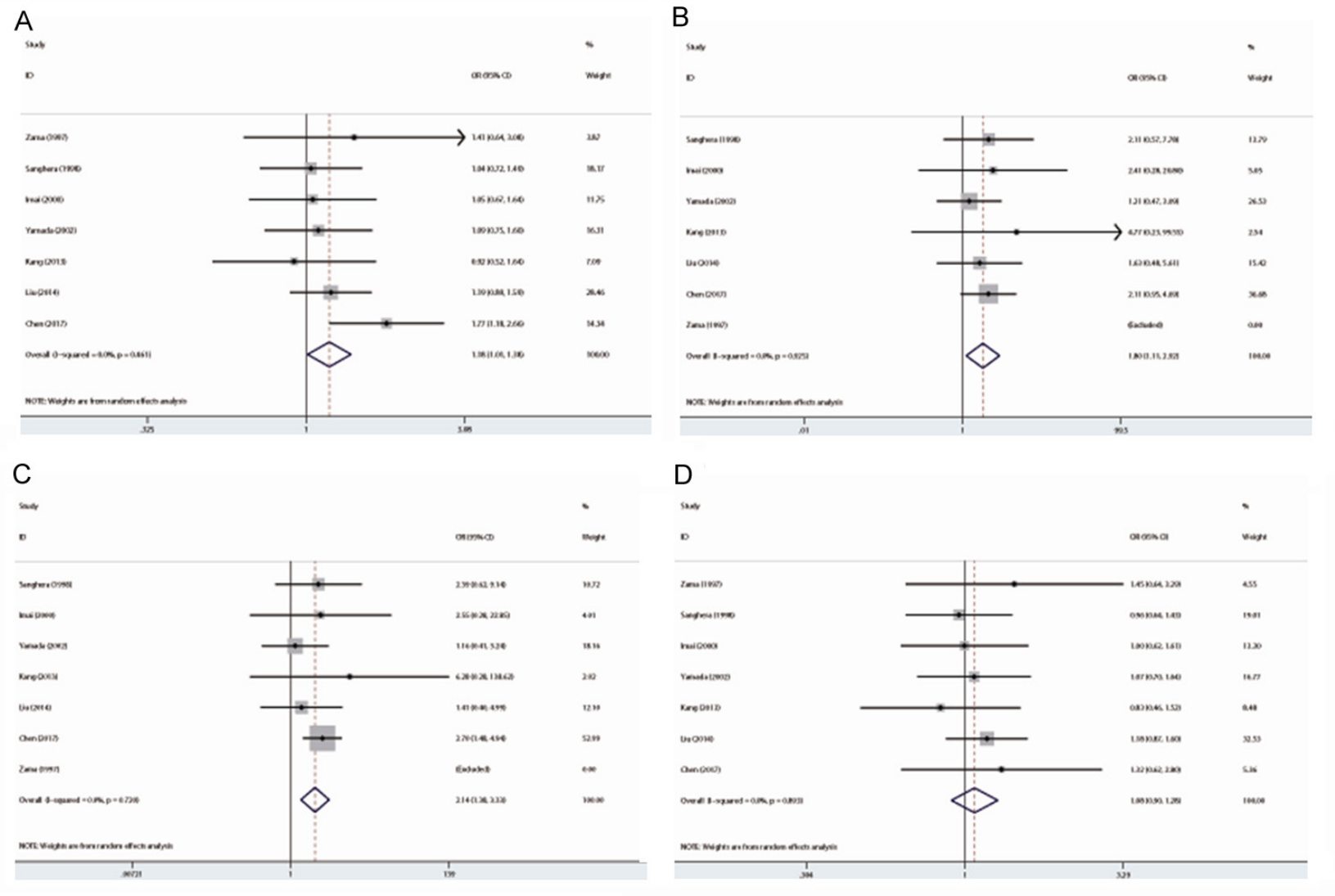
Figure 2. Meta-analysis on the association of PON1 L55M polymorphisms and CHD risk in the overall population. A. Allele model: L vs. M. B. Homozygote model: LL vs. MM. C. Heterozygote model: LM vs. MM. D. Recessive model: LL vs. LM+MM. E. Dominant model: LL+LM vs. MM. F. Funnel plot of publication bias with pseudo 95% confidence limit in allelic model. CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.



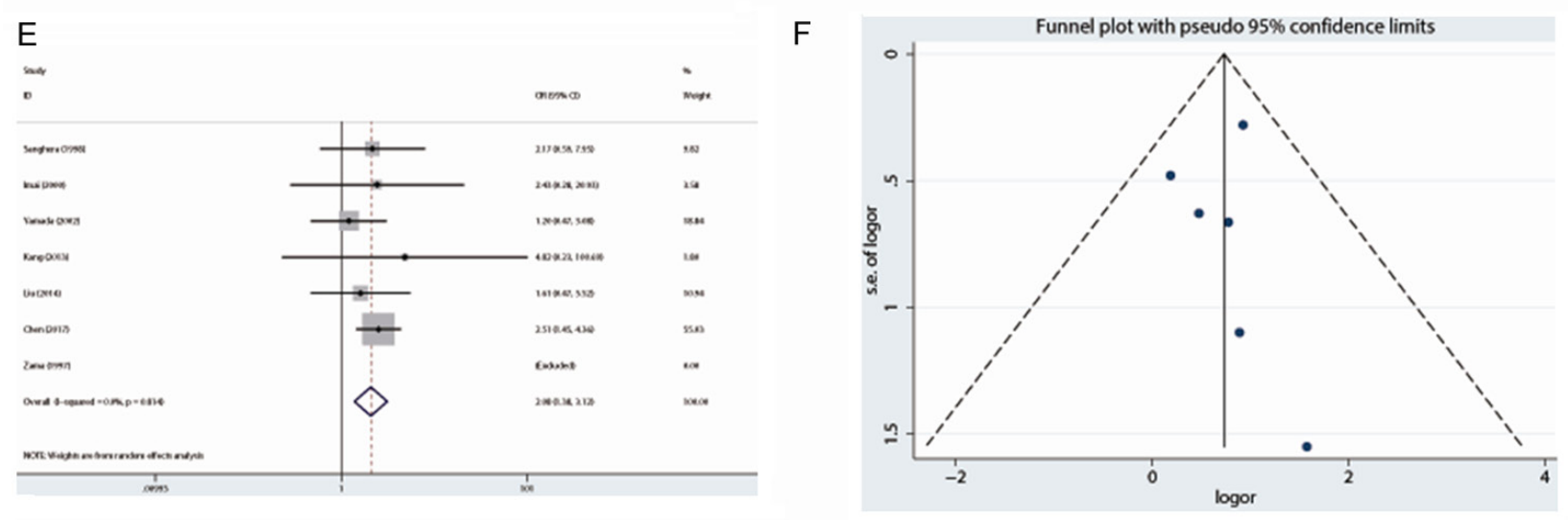


Figure 3. Subgroup analysis: Association of PON1 L55M polymorphisms and CHD risk in Asian. A. Allele model: L vs. M. B. Homozygote model: LL vs. MM. C. Heterozygote model: LM vs. MM. D. Recessive model: LL vs. LM+MM. E. Dominant model: LL+LM vs. MM. F. Funnel plot of publication bias with pseudo 95% confidence limit in allelic model. CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.

Table 4. Subgroup analyses of association between PON1 L55M polymorphism and CHD

Genetic model	Subgroup	Number	OR (95% CI)	I^2 (%)	*P* value
Allelic			1.18 (1.01, 1.38))	0	0.035
Homozygote			1.80 (1.11, 2.92)	0	0.017
Heterozygote	Asian	7	2.14 (1.38, 3.33)	0	0
Recessive			1.08 (0.91, 1.21)	0	0.404
Dominant			2.08 (1.38, 3.12)	0	0
Allelic			1.08 (1.00, 2.00)	64	0.045
Homozygote			1.16 (0.98, 1.38)	60	0.082
Heterozygote	Caucasian	39	1.14 (0.98, 1.32)	54	0.088
Recessive			1.16 (0.97, 1.16)	45	0.184
Dominant			1.16 (1.00, 1.36)	61	0.105
Allelic			1.11 (1.01, 1.21)	66	0.023
Homozygote			1.28 (1.04 , 1.57)	62	0.018
Heterozygote	Not MI	37	1.25 (1.03, 1.52)	60	0.024
Recessive			1.07 (0.98, 1.17)	40	0.143
Dominant			1.28 (1.05, 1.56)	55	0.015
Allelic			1.03 (0.94, 1.10)	0	0.746
Homozygote			0.97 (0.81, 1.15)	0	0.696
Heterozygote	MI	13	1.03 (0.89, 1.20)	0	0.686
Recessive			1.02 (0.91, 1.14)	7	0.758
Dominant			1.02 (0.88, 1.17)	0	0.833
Allelic			1.18 (1.01, 1.40)	59	0.038
Homozygote			1.45 (1.05, 2.00)	50	0.022
Heterozygote	N<500	22	1.49 (1.10, 2.01)	53	0.009
Recessive			1.07 (0.98, 1.17)	55	0.143
Dominant			1.28 (1.05, 1.56)	58	0.015
Allelic			1.05 (0.97, 1.07)	15	0.468
Homozygote			1.01 (0.88, 1.15)	27	0.919
Heterozygote	N ≥ 500	24	0.99 (0.89, 1.12)	8	0.877
Recessive			1.02 (0.91, 1.14)	0	0.758
Dominant			1.02 (0.88, 1.17)	20	0.833
Allelic			1.04 (0.97, 1.10)	38	0.27
Homozygote			1.07 (0.93, 1.28)	32	0.354
Heterozygote	HWE > 0.05	32	1.05 (0.94, 1.16)	8	0.41
Recessive			1.03 (0.95, 1.12)	36	0.502
Dominant			1.06 (0.95, 1.19)	21	0.3
Allelic			1.26 (1.03, 1.54)	76	0.024
Homozygote			1.63 (1.01, 2.64)	73	0.046
Heterozygote	HWE ≤ 0.05	14	1.59 (0.97, 2.59)	77	0.064
Recessive			1.16 (0.99, 1.35)	28	0.063
Dominant			1.63 (1.01, 2.63)	78	0.046

The current meta-analysis consolidated 46 eligible studies concerning the relationship between PON1 L55M polymorphisms and the risk of CHD. Pooled analysis showed no significant association between L55M polymorphisms and CHD. However, subgroup analysis, performed by ethnicity, showed this association was significant in the Asian population under four genetic models. However, the association was still insignificant among Caucasians, indicating that ethnicity differences had a significant impact on the polymorphism ef-

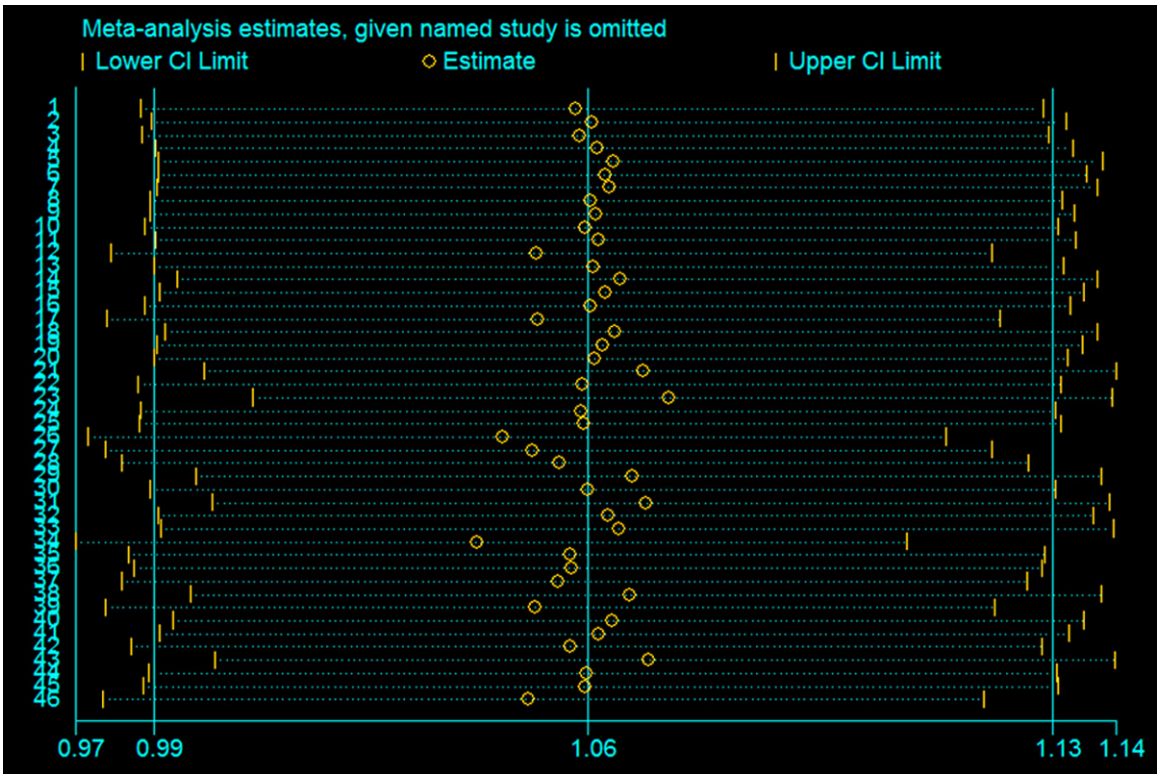


Figure 4. Sensitivity analysis of pooled OR coefficients concerning the relationship between PON1 L55M polymorphisms and CHD risk. CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.

fects and that Asians are more susceptible to PON1 polymorphisms in occurrence of CHD. According to subgroup analysis by diagnoses (MI or not), the gene susceptibility of L55M in patients with MI seemed weaker than that in CHD patients without MI. Compared to chronic coronary artery diseases, which are characterized by progressive coronary stenosis and more influenced by serum activity of PON1 [44], myocardial infarction is a much more acute progress with other uncertain factors participating, such as plaque rupture, thrombosis, or even coronary spasms [37]. The influence of these complex factors may weaken the impact of PON1 activity on MI, thus weakening the association of PON1 L55M polymorphisms with MI. To further confirm the results of total analysis, subgroup analyses, stratified by sample size and results of HWE testing, were conducted. In groups of n > 500 and p of HWE testing > 0.05, which represent a comparatively higher quality of included studies, synthesized results were consistent with the overall analysis. Although subgroups of studies with n<500 and p of HWE testing ≤ 0.05 drew statistically significant results, the comparatively low quality of these studies made this result less convincing.

Results of the current study found are generally consistent with the meta-analysis by Hernandez-Diaz, Y et al. Both studies found that the association between PON1 L55M polymorphisms and CHD is insignificant in the overall population, but significant in Asians (allelic, heterozygote, and recessive models). According to subgroup-analysis of Asian populations, four gene contrast models drew significant results, reinforcing the conclusion that Asians are more genetically susceptible to CHD in the gene of PON1 L55M. There were also some differences between the meta-analysis of Hernandez-Diaz, Y et al. and the current study. They concluded that the genetic susceptibility for CHD is associated with PON1 L55M polymorphisms in Europeans (from recessive model). However, the current study deemed their study less rigorous because only one gene model contrast model yielded a significant association. In contrast, the current study found no association between PON1 L55M polymorphisms and CHD

in Caucasians (from all five models). Differences in PON1 L55M susceptibility for CHD may stem from huge differences in diet and lifestyles between Asians and Caucasians. Therefore, present results should be viewed as an update and revision to existing knowledge in this field.

Previously, two large meta-analyses revealed a significant correlation between PON1 activity and risk of CHD [63, 64]. However, whether PON1 is related to CHD at the gene level remains controversial. The current meta-analysis provides insight into the relationship between PON1 L55M polymorphisms and risk of CHD. In contrast to the active substance levels, no significant association between PON1 L55M polymorphisms and CHD was found. Reason for this difference may be that not all genetic abnormalities lead to decreased PON1 activity. Thus, it does not affect the oxidation modification process of LDL in atherosclerosis.

The main strength of the current study is the size. It is the largest meta-analysis, by far, concerning the relationship between PON1 L55M polymorphisms and risk of CHD. Moreover, this study specifically conducted subgroup analyses by excluding small studies with n<500 and low-quality studies which failed to fit HWE testing. Results did not change. However, the current meta-analysis does have some limitations. First, 14 studies did not fit HWE testing in the control group, though results were not altered after the omission of these studies. Second, this study did not include African populations. Although three of the studies were from African countries (Tunisia and Morocco) [14, 39, 54], the subjects involved were Caucasians. Third, significant inter-study heterogeneity existed in many of the comparisons. This may have interfered with the interpretation of current findings. Heterogeneity may have arised from differences in age distribution, gender ratio, CHD phenotypes, prevalence of diet, and lifestyle factors.

Conclusion

Taken together, the current meta-analysis concludes that L55M polymorphisms in PON1 genes are not associated with risk of CHD in the overall population. However, this association is significant in Asians. Subgroup analyses of small studies and low-quality studies yield significant results, lowering the credibility of positive association between PON1 L55M polymorphisms and CHD. Therefore, more high-quality case-control studies are necessary to further validate the association between PON1 L55M polymorphisms and the risk of CHD.

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Disclosure of conflict of interest

None.

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References

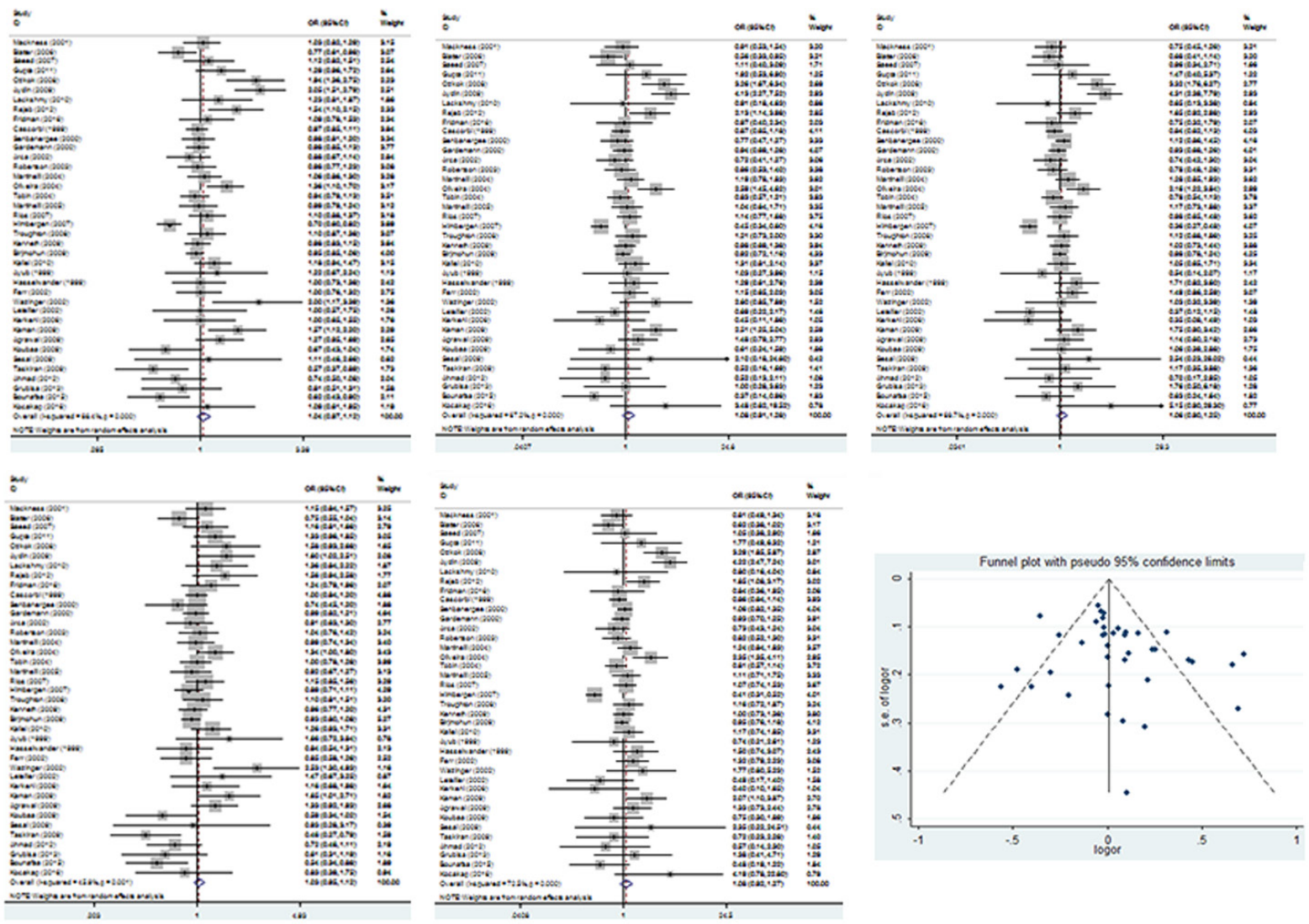
[1] Ferrari R and Fox K. Heart rate reduction in coronary artery disease and heart failure. Nat Rev Cardiol 2016; 13: 493-501.

[2] Newton JN, Briggs AD, Murray CJ, Dicker D, Foreman KJ, Wang H, Naghavi M, Forouzanfar MH, Ohno SL, Barber RM, Vos T, Stanaway JD, Schmidt JC, Hughes AJ, Fay DF, Ecob R, Gresser C, McKee M, Rutter H, Abubakar I, Ali R, Anderson HR, Banerjee A, Bennett DA, Bernabé E, Bhui KS, Biryukov SM, Bourne RR, Brayne CE, Bruce NG, Brugha TS, Burch M, Capewell S, Casey D, Chowdhury R19, Coates MM3, Cooper C20, Critchley JA, Dargan PI, Dherani MK, Elliott P, Ezzati M, Fenton KA, Fraser MS, Fürst T, Greaves F, Green MA, Gunnell DJ, Hannigan BM, Hay RJ, Hay SI, Hemingway H, Larson HJ, Looker KJ, Lunevicius R, Lyons RA, Marcenes W, Mason-Jones AJ, Matthews FE, Moller H, Murdoch ME, Newton CR, Pearce N, Piel FB, Pope D, Rahimi K, Rodriguez A, Scarborough P, Schumacher AE, Shiue I, Smeeth L, Tedstone A, Valabhji J, Williams HC, Wolfe CD, Woolf AD and Davis AC. Changes in health in England, with analysis by English regions and areas of deprivation, 1990-2013: a systematic analysis for the global burden of disease study 2013. Lancet 2015; 386: 2257-2274.

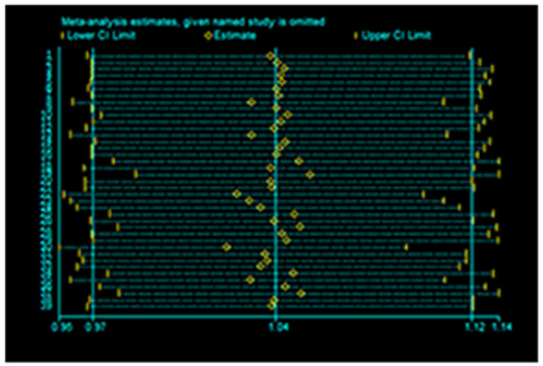
[3] Mineo C and Shaul PW. PON-dering differences in HDL function in coronary artery disease. J Clin Invest 2011; 121: 2545-2548.
[4] Durmaz T, Keles T, Ayhan H, Cay N, Bayram NA, Bilen E, Akcay M, Erdogan KE, Erel O and Bozkurt E. Diminished serum paraoxonase activity in patients with coronary artery calcification. Kardiol Pol 2014; 72: 831-838.
[5] Mackness M, Durrington P and Mackness B. 3SY05-6 paraoxonase: biochemistry and contribution to atherosclerosis. Atherosclerosis Supplements 2003; 4: 177-178.
[6] Lawlor DA, Day IN, Gaunt TR, Hinks LJ, Briggs PJ, Kiessling M, Timpson N, Smith GD, Ebrahim S. The association of the PON1 Q192R polymorphism with coronary heart disease: findings from the British Women's heart and health cohort study and a meta-analysis. BMC Genetics 2004; 5: 17.
[7] Zhao Y, Ma Y, Fang Y, Liu L, Wu S, Fu D and Wang X. Association between PON1 activity and coronary heart disease risk: a meta-analysis based on 43 studies. Mol Genet Metab 2012; 105: 141-148.
[8] Ko YL, Ko YS, Wang SM, Hsu LA, Chang CJ, Chu PH, Cheng NJ, Chen WJ, Chiang CW, Lee YS. The Gln-Arg 191 polymorphism of the human paraoxonase gene is not associated with the risk of coronary artery disease among Chinese in Taiwan. Atherosclerosis 1998; 141: 259-264.
[9] Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ and Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nature Genetics 1993; 3: 73.
[10] Wang M, Lang X, Zou L, Huang S and Xu Z. Four genetic polymorphisms of paraoxonase gene and risk of coronary heart disease: a meta-analysis based on 88 case-control studies. Atherosclerosis 2011; 214: 377-385.
[11] Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop IE, González-Castro TB, Rodríguez-Pérez C, López-Narváez ML, Rodríguez-Pérez JM, Cámara-Álvarez JF. Effects of paraoxonase 1 gene polymorphisms on heart diseases Systematic review and meta-analysis of 64 case-control studies. Medicine (Baltimore) 2016; 95: e5298.
[12] Zhang Z, Ou J, Cai P, Niu B and Li J. Association between the PON1 Q192R polymorphism and coronary heart disease in Chinese A meta-analysis. Medicine (Baltimore) 2018; 97: e11151.
[13] Wheeler JG, Keavney BD, Watkins H, Collins R and Danesh J. Four paraoxonase gene polymorphisms in 11,212 cases of coronary heart disease and 12,786 controls: meta-analysis of 43 studies. Lancet 2004; 363: 689-695.
[14] Bounafaa A, Berrougui H, Ghalim N, Nasser B, Bagri A, Moujahid A, Ikhlef S, Camponova P, Yamoul N, Simo OK, Essamadi A and Khalil A. Association between paraoxonase 1 (PON1) polymorphisms and the risk of acute coronary syndrome in a north african population. PLoS One 2015; 10: e0133719.
[15] Chen H, Ding S, Zhou M, Wu X, Liu X, Liu J, Wu Y and Liu D. PON1 L55M and Q192R gene polymorphisms and CAD risks in patients with hyperlipidemia : clinical study of possible associations. Herz 2018; 43: 642-648.
[16] Fridman O, Gariglio L, Riviere S, Porcile R, Fuchs A and Potenzoni M. Paraoxonase 1 gene polymorphisms and enzyme activities in coronary artery disease and its relationship to serum lipids and glycemia. Arch Cardiol Mex 2016; 86: 350-357.
[17] Sayın Kocakap DB, Doğru MT, Şimşek V, Çabuk F, Yıldırım N, Çelik Y, Alyılmaz Bekmez S, Erdem S. The association of paraoxonase 1 gene L55M polymorphism with the extent and severity of coronary artery disease in the Turkish population and its dependence on gender. Anatol J Cardiol 2016; 16: 175-182.
[18] Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. Int J Surg 2010; 8: 336-41.
[19] Stang A. Critical evaluation of the newcastle-ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-605.
[20] Zama T, Murata M, Matsubara Y, Kawano K, Aoki N, Yoshino H, Watanabe G, Ishikawa K and Ikeda Y. A 192Arg variant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. Arterioscler Thromb Vasc Biol 1997; 17: 3565-3569.
[21] Sanghera DK, Saha N and Kamboh MI. The codon 55 polymorphism in the paraoxonase 1 gene is not associated with the risk of coronary heart disease in Asian Indians and Chinese. Atherosclerosis 1998; 136: 217-223.
[22] Ayub A, Mackness MI, Arrol S, Mackness B, Patel J and Durrington PN. Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol 1999; 19: 330-335.
[23] Hasselwander O, Savage DA, McMaster D, Loughrey CM, McNamee PT, Middleton D, Nicholls DP, Maxwell AP and Young IS. Paraoxonase polymorphisms are not associated with cardiovascular risk in renal transplant recipients. Kidney Int 1999; 56: 289-298.
[24] Cascorbi I, Laule M, Mrozikiewicz PM, Mrozikiewicz A, Andel C, Baumann G, Roots I and Stangl K. Mutations in the human paraoxonase 1 gene: frequencies, allelic linkages, and asso-

ciation with coronary artery disease. Pharmacogenetics 1999; 9: 755-761.
[25] Sen-Banerjee S, Siles X and Campos H. Tobacco smoking modifies association between Gln-Arg192 polymorphism of human paraoxonase gene and risk of myocardial infarction. Arterioscler Thromb Vasc Biol 2000; 20: 2120-2126.
[26] Gardemann A, Philipp M, Hess K, Katz N, Tillmanns H and Haberbosch W. The paraoxonase Leu-Met54 and Gln-Arg191 gene polymorphisms are not associated with the risk of coronary heart disease. Atherosclerosis 2000; 152: 421-431.
[27] Imai Y, Morita H, Kurihara H, Sugiyama T, Kato N, Ebihara A, Hamada C, Kurihara Y, Shindo T, Oh-hashi Y, Yazaki Y. Evidence for association between paraoxonase gene polymorphisms and atherosclerotic diseases. Atherosclerosis 2000; 149: 435-42.
[28] Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN and Mackness MI. Paraoxonase status in coronary heart disease - are activity and concentration more important than genotype? Arterioscler Thromb Vasc Biol 2001; 21: 1451-1457.
[29] Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M and Yokota M. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 2002; 347: 1916-1923.
[30] Ferre N, Tous M, Paul A, Zamora A, Vendrell JJ, Bardaji A, Camps J, Richart C and Joven J. Paraoxonase Gln-Arg(192) and Leu-Met(55) gene polymorphisms and enzyme activity in a population with a low rate of coronary heart disease. Clin Biochem 2002; 35: 197-203.
[31] Watzinger N, Schmidt H, Schumacher M, Schmidt R, Eber B, Fruhwald FM, Zweiker R, Kostner GM and Klein W. Human paraoxonase 1 gene polymorphisms and the risk of coronary heart disease: a community-based study. Cardiology 2002; 98: 116-122.
[32] Letellier C, Durou MR, Jouanolle AM, Le Gall JY, Poirier JY and Ruelland A. Serum paraoxonase activity and paraoxonase gene polymorphism in type 2 diabetic patients with or without vascular complications. Diabetes Metab 2002; 28: 297-304.
[33] Arca M, Ombres D, Montali A, Campagna F, Mangieri E, Tanzilli G, Campa PP, Ricci G, Verna R and Pannitteri G. PON1 L55M polymorphism is not a predictor of coronary atherosclerosis either alone or in combination with Q192R polymorphism in an Italian population. Eur J Clin Invest 2002; 32: 9-15.
[34] Robertson KS, Hawe E, Miller GJ, Talmud PJ and Humphries SE. Human paraoxonase gene cluster polymorphisms as predictors of coronary heart disease risk in the prospective Northwick Park Heart Study II. Biochim Biophys Acta 2003; 1639: 203-212.
[35] Martinelli N, Girelli D, Olivieri O, Stranieri C, Trabetti E, Pizzolo F, Friso S, Tenuti I, Cheng S, Grow MA, Pignatti PF and Corrocher R. Interaction between smoking and PON2 Ser(311)Cys polymorphism as a determinant of the risk of myocardial infarction. Eur J Clin Invest 2004; 34: 14-20.
[36] Oliveira SA, Mansur AP, Ribeiro CC, Ramires JAF and Annichino-Bizzacchi JM. PON1 M/L55 mutation protects high-risk patients against coronary artery disease. Int J Cardiol 2004; 94: 73-77.
[37] Tobin MD, Braund PS, Burton PR, Thompson JR, Steeds R, Channer K, Cheng S, Lindpaintner K, Samani NJ. Genotypes and haplotypes predisposing to myocardial infarction: a multilocus case-control study. Eur Heart J 2004; 459-467.
[38] Martinelli N, Girelli D, Olivieri O, Cavallari U, Biscuola M, Trabetti E, Friso S, Pizzolo F, Tenuti I, Bozzini C, Villa G, Ceradini B, Sandri M, Cheng S, Grow MA, Pignatti PF and Corrocher R. Interaction between metabolic syndrome and PON1 polymorphisms as a determinant of the risk of coronary artery disease. Clin Exp Med 2005; 5: 20-30.
[39] Kerkeni M, Addad F, Chauffert M, Chuniaud L, Miled A, Trivin F and Maaroufi K. Hyperhomocysteinemia, paraoxonase activity and risk of coronary artery disease. Clin Biochem 2006; 39: 821-825.
[40] Blatter Garin MC, Moren X and James RW. Paraoxonase-1 and serum concentrations of HDL-cholesterol and apoA-I. J Lipid Res 2006; 47: 515-520.
[41] Rios DL, D'Onofrio LO, Cerqueira CC, Bonfim-Silva R, Carvalho HG, Santos-Filho A and Galvao-Castro B. Paraoxonase 1 gene polymorphisms in angiographically assessed coronary artery disease: evidence for gender interaction among Brazilians. Clin Chem Lab Med 2007; 45: 874-878.
[42] van Himbergen TM, van der Schouw YT, Voorbij HA, van Tits LJ, Stalenhoef AF, Peeters PH, Roest M. Paraoxonase (PON1) and the risk for coronary heart disease and myocardial infarction in a general population of Dutch women. Atherosclerosis 2008; 199: 408-414.
[43] Saeed M, Iqbal MP, Yousuf FA, Perveen S, Shafiq M, Sajid J and Frossard PM. Interactions and associations of paraoxonase gene cluster polymorphisms with myocardial infarction in a Pakistani population. Clin Genet 2007; 71: 238-244.
[44] Troughton JA, Woodside JV, Yarnell JW, Arveiler D, Amouyel P, Ferrières J, Ducimetière P, Patterson CC, Luc G; PRIME Study Group. Paraoxonase activity and coronary heart disease risk

in healthy middle-aged males: The PRIME study. Atherosclerosis 2008; 197: 556-563.
[45] Ozkök E, Aydin M, Babalik E, Ozbek Z, Ince N, Kara I. Combined impact of matrix metalloproteinase-3 and paraoxonase 1 55/192 gene variants on coronary artery disease in Turkish patients. Med Sci Monit 2008; 14: CR536-42.
[46] Kaman D, Ilhan N, Metin K, Akbulut M, Ustündağ B. A preliminary study of human paraoxonase and PON 1 L/M55-PON 1 Q/R 192 polymorphisms in Turkish patients with coronary artery disease. Cell Biochem Funct 2009; 27: 88-92.
[47] Agrawal S, Tripathi G, Prajnya R, Sinha N, Gilmour A, Bush L and Mastana S. Paraoxonase 1 gene polymorphisms contribute to coronary artery disease risk among north Indians. Indian J Med Sci 2009; 63: 335-344.
[48] Koubaa N, Nakbi A, Hammami S, Attia N, Mehri S, Ben Hamda K, Ben Farhat M, Miled A, Hammami M. Association of homocysteine thiolactonase activity and PON1 polymorphisms with the severity of acute coronary syndrome. Clin Biochem 2009; 42: 771-6.
[49] Sesal C, Ciloglu F, Peker I and Sayar N. Role of angiotensin converting enzyme, paraoxonase 1 55, 192 gene polymorphisms in syndrome X and coronary heart disease. Pak J Biol Sci 2009; 12: 46-51.
[50] Taskiran P, Cam SF, Sekuri C, Tuzun N, Alioglu E, Altintas N and Berdeli A. [The relationship between paraoxanase gene Leu-Met (55) and Gln-Arg (192) polymorphisms and coronary artery disease]. Turk Kardiyol Dern Ars 2009; 37: 473-478.
[51] Mukamal KJ, Pai JK and Jensen MK. Paraoxonase 1 polymorphisms and Risk of Myocardial Infarction in Women and Men. Circ J 2009; 73: 1302-7.
[52] Birjmohun RS, Vergeer M, Stroes ES, Sandhu MS, Ricketts SL, Tanck MW, Wareham NJ, Jukema JW, Kastelein JJ, Khaw KT and Boekholdt SM. Both paraoxonase-1 genotype and activity do not predict the risk of future coronary artery disease; the EPIC-Norfolk Prospective Population Study. PLoS One 2009; 4: e6809.
[53] Aydin M, Gokkusu C, Ozkok E, Tulubas F, Unlucerci Y, Pamukcu B, Ozbek Z and Umman B. Association of genetic variants in methylenetetrahydrofolate reductase and paraoxonase-1 genes with homocysteine, folate and vitamin B12 in coronary artery disease. Mol Cell Biochem 2009; 325: 199-208.
[54] Kallel A, Sediri Y, Sbai MH, Mourali MS, Feki M, Elasmi M, Taieb SH, Sanhaji H, Souheil O, Mechmeche R, Jemaa R and Kaabachi N. The paraoxonase L55M and Q192R gene polymorphisms and myocardial infarction in a Tunisian population. Clin Biochem 2010; 43: 1461-1463.
[55] Lakshmy R, Ahmad D, Abraham RA, Sharma M, Vemparala K, Das S, Reddy KS and Prabhakaran D. Paraoxonase gene Q192R & L55M polymorphisms in Indians with acute myocardial infarction & association with oxidized low density lipoprotein. Indian J Med Res 2010; 131: 522-529.
[56] Gupta N, Singh S, Maturu VN, Sharma YP and Gill KD. Paraoxonase 1 (PON1) polymorphisms, haplotypes and activity in predicting cad risk in North-West Indian Punjabis. PLoS One 2011; 6: e17805.
[57] Ahmad I, Narang R, Venkatraman A and Das N. Two-and three-locus haplotypes of the paraoxonase (PON1) gene are associated with coronary artery disease in Asian Indians. Gene 2012; 506: 242-247.
[58] Rejeb J, Omezzine A, Rebhi L, Boumaiza I, Mabrouk H, Rhif H, Ben Rejeb N, Nabli N, Douki W, Ben Abdelaziz A, Boughzala E and Bouslama A. Association of PON1 and PON2 polymorphisms with PON1 activity and significant coronary stenosis in a Tunisian population. Biochem Genet 2013; 51: 76-91.
[59] Grubisa I, Otasevic P, Dimkovic N, Nedeljkovic I, Toljic B and Vucinic N. Genetic polymorphisms of paraoxonase 1 and susceptibility to atherogenesis. Srp Arh Celok Lek 2013; 141: 629-633.
[60] Kang YH1, Lao HY, Wu H, Lai WH, Li XX, Yu XY, Chen JY, Zhong SL. Association of PON1 genotype and haplotype with susceptibility to coronary artery disease and clinical outcomes in dual antiplatelet-treated Han Chinese patients. European Eur J Clin Pharmacol 2013; 69: 1511-1519.
[61] Liu T, Zhang X, Zhang J, Liang Z, Cai W, Huang M, Yan C, Zhu Z and Han Y. Association between PON1 rs662 polymorphism and coronary artery disease. Eur J Clin Nutr 2014; 68: 1029-1035.
[62] Sanghera DK, Saha N, Aston CE and Kamboh MI. Genetic polymorphism of paraoxonase and the risk of coronary heart disease. Arterioscler Thromb Vasc Biol 1997; 17: 1067-1073.
[63] Wang M, Lang X, Cui S, Zou L, Cao J, Wang S and Wu X. Quantitative assessment of the influence of paraoxonase 1 activity and coronary heart disease risk. DNA Cell Biol 2012; 31: 975-982.
[64] Zhao Y, Ma Y, Fang Y, Liu L, Wu S, Fu D and Wang X. Association between PON1 activity and coronary heart disease risk: a meta-analysis based on 43 studies. Mol Genet Metab 2012; 105: 141-148.



Supplementary Figure 1. Subgroup analysis of association of PON1 L55M polymorphisms and CHD Caucasians.



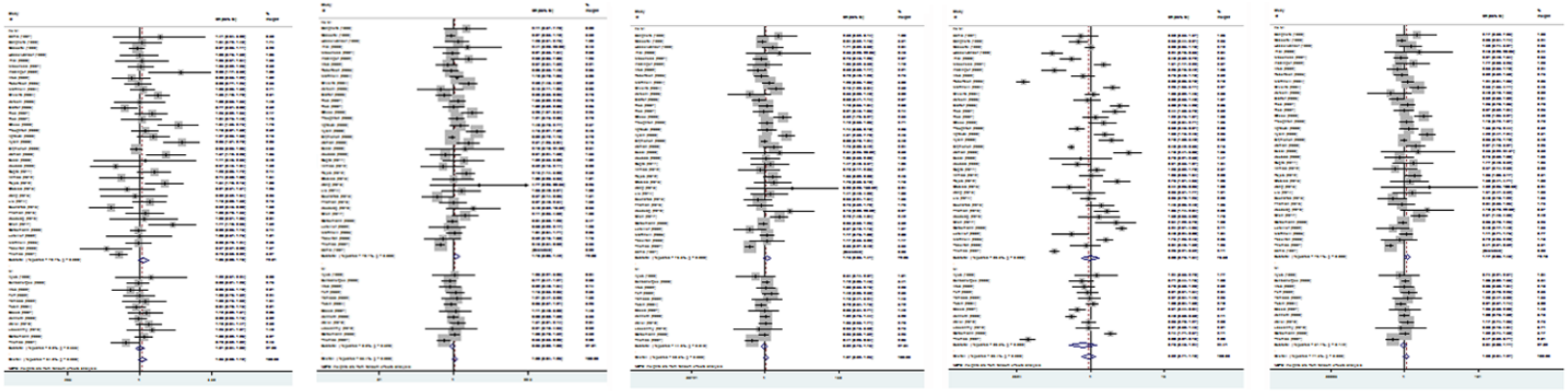
Begg's Test

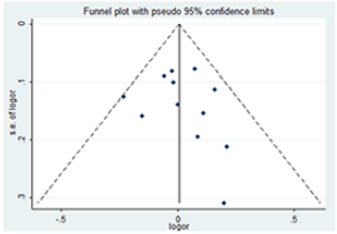
adj. Kendall's Score (P-Q) =	119
Std. Dev. of Score =	82.67
Number of Studies =	39
z =	1.44
Pr > |z| =	0.150
z =	1.43 (continuity corrected)
Pr > |z| =	0.153 (continuity corrected)

Egger's test

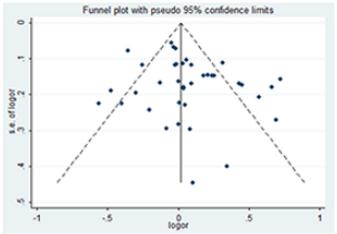
Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.1163137	.0821337	-1.42	0.165	-.2827324	.050105
bias	1.103872	.6710123	1.65	0.108	-.2557278	2.463472

Supplementary Figure 2. Subgroup analysis by diagnosis: Association between PON1 L55M polymorphisms and CHD risk.





funel plot-MI



funel plotnot-not MI

Begg's Test

adj. Kendall's Score (P-Q) =	10
Std. Dev. of Score =	14.58
Number of Studies =	12
z =	0.69
Pr > |z| =	0.493
z =	0.62 (continuity corrected)
Pr > |z| =	0.537 (continuity corrected)

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.0473953	.0964197	-0.49	0.634	-.2622358	.1674371
bias	.4858247	.8168456	0.59	0.565	-1.334221	2.30587

Begg and Egger' s test-MI

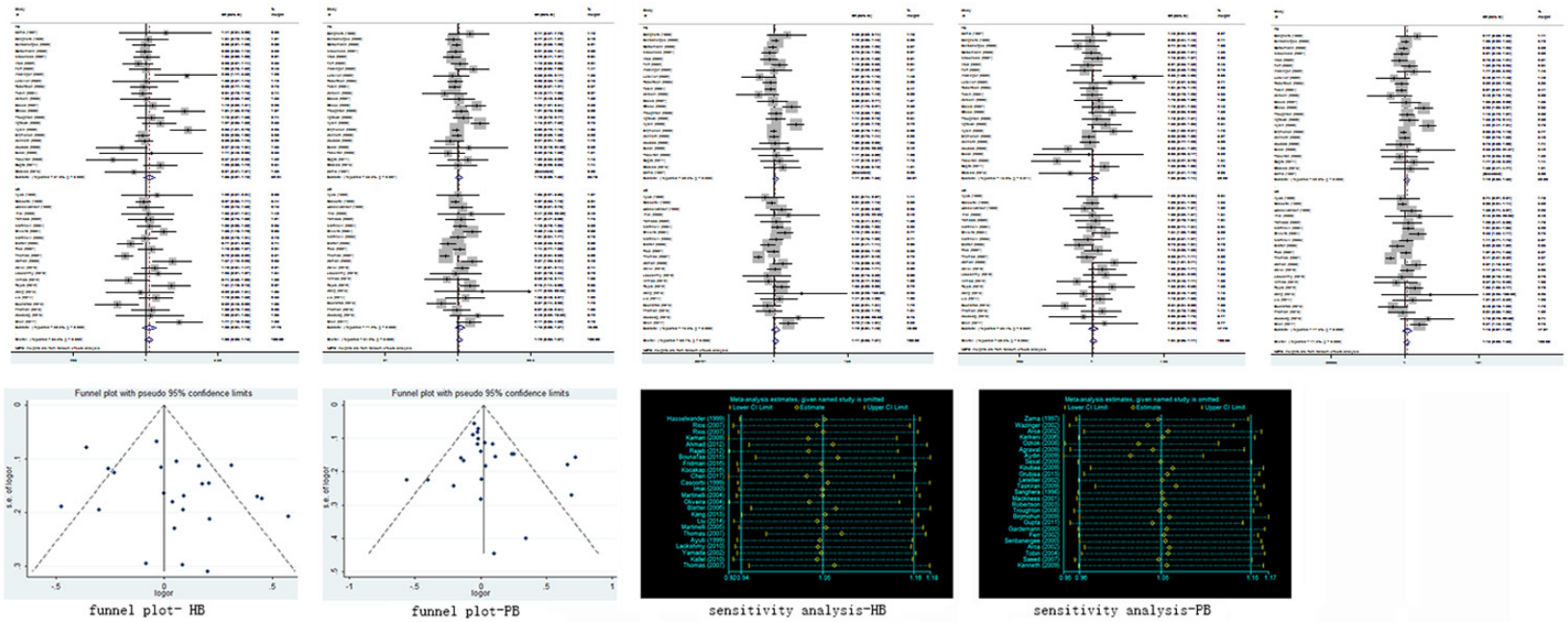
Begg's Test

adj. Kendall's Score (P-Q) =	51
Std. Dev. of Score =	79.54
Number of Studies =	38
z =	0.64
Pr > |z| =	0.521
z =	0.63 (continuity corrected)
Pr > |z| =	0.530 (continuity corrected)

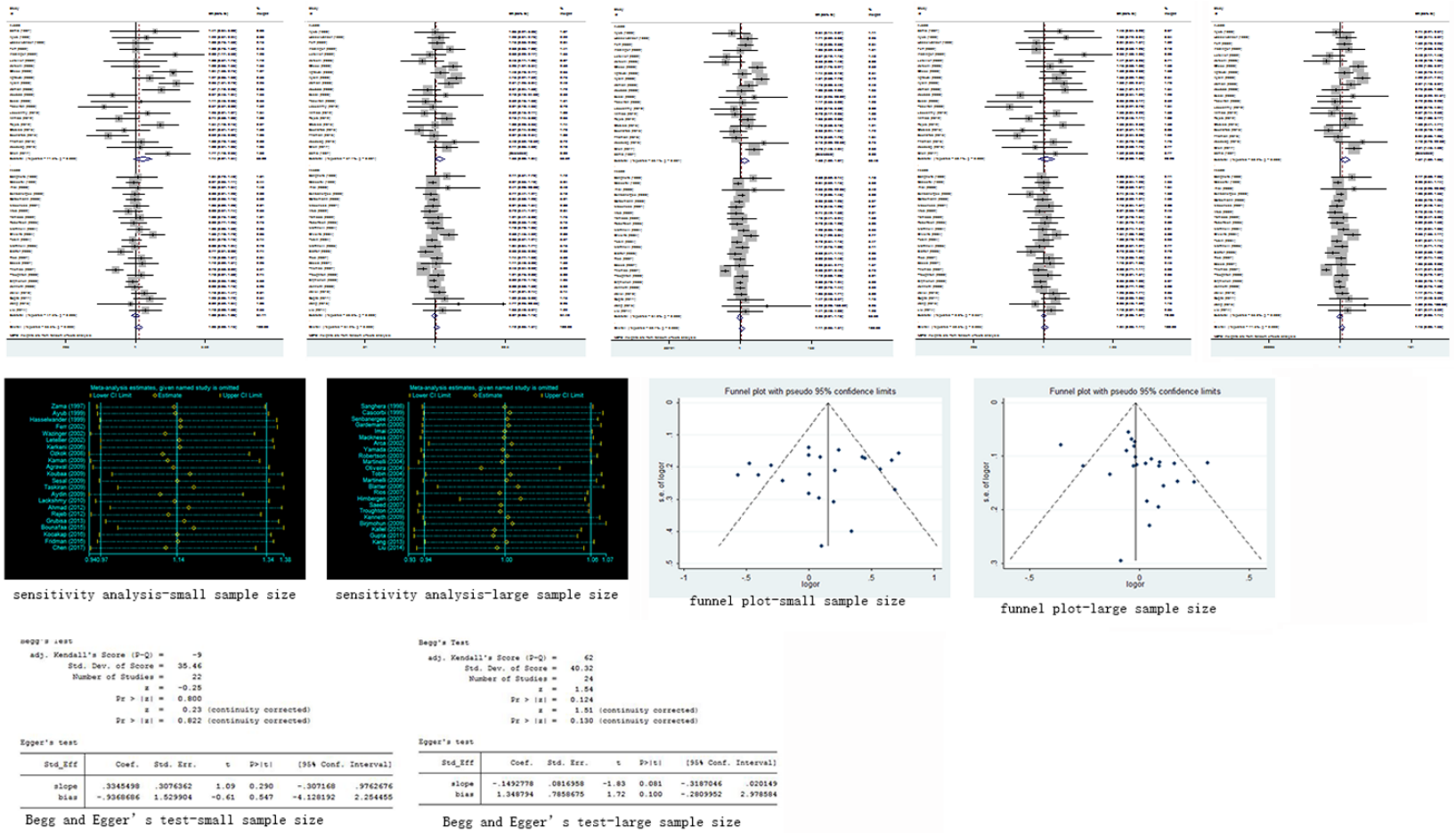
Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.114138	.0874404	-1.31	0.200	-.2914752	.0631993
bias	1.097858	.6570241	1.67	0.103	-.2346488	2.430364

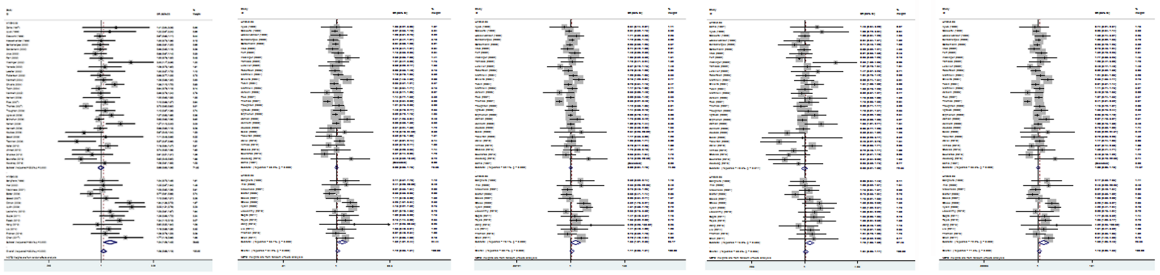
Begg and Egger' s test-not MI



Supplementary Figure 3. Subgroup analysis by source of control: Association of PON1 L55M polymorphisms and CHD risk.



Supplementary Figure 4. Subgroup analysis by sample size: Association of PON1 L55M polymorphisms and CHD risk.



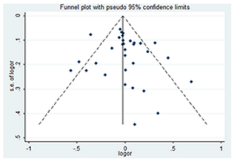
Begg's Test

adj. Kendall's Score (P-Q)	62
Std. Dev. of Score	61.67
Number of Studies	32
z	1.01
Pr > |z|	0.315
z	0.99 (continuity corrected)
Pr > |z|	0.323 (continuity corrected)

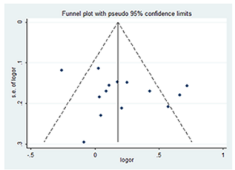
Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.0755245	.0704511	-1.07	0.292	-.2194049	.0683559
bias	.4948668	.5890121	0.84	0.407	-.7080563	1.69779

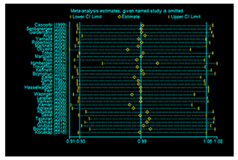
Begg and Egger's test- HWE meet



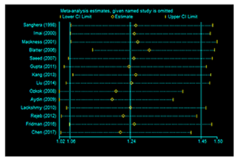
funnel plot-HWE meet



funnel plot- HWE not meet



sensitivity analysis-HWE meet



sensitivity analysis-HWE not meet

Begg's Test

adj. Kendall's Score (P-Q)	5
Std. Dev. of Score	18.27
Number of Studies	14
z	0.27
Pr > |z|	0.784
z	0.22 (continuity corrected)
Pr > |z|	0.827 (continuity corrected)

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.2142184	.3337131	-0.64	0.533	-.9413168	.5128801
bias	2.486486	2.049643	1.21	0.248	-1.979302	6.952274

Begg and Egger's test- HWE not meet

Supplementary Figure 5. Subgroup analysis by HWE test: association between PON1 L55M polymorphisms and CHD risk.

Meta-analysis

Method	Pooled Est	95% CI Lower	95% CI Upper	Asymptotic z_value	Asymptotic p_value	No. of studies
Fixed	0.004	-0.004	0.011	0.946	0.344	46
Random	0.009	-0.007	0.025	1.059	0.290	

Test for heterogeneity: Q= 126.169 on 45 degrees of freedom (p= 0.000)
Moment-based estimate of between studies variance = 0.001

Trimming estimator: Linear
Meta-analysis type: Random-effects model

iteration	estimate	Tn	# to trim	diff
1	0.009	596	2	1081
2	0.005	634	4	76
3	-0.000	687	6	106
4	-0.003	716	8	58
5	-0.005	741	9	50
6	-0.006	753	9	24
7	-0.006	753	9	0

Filled
Meta-analysis

Method	Pooled Est	95% CI Lower	95% CI Upper	Asymptotic z_value	Asymptotic p_value	No. of studies
Fixed	-0.002	-0.009	0.006	-0.437	0.662	55
Random	-0.010	-0.028	0.008	-1.042	0.297	

Test for heterogeneity: Q= 205.792 on 54 degrees of freedom (p= 0.000)
Moment-based estimate of between studies variance = 0.002

Supplementary Figure 6. Results of trim and fill method of overall analysis.