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META-ANALYSIS

Relationship between Paraoxonase 1 (PON1) gene polymorphisms and susceptibility of stroke: a meta-analysis

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Abstract Genetic variants of paraoxonase 1 (*PON1*) were implicated in stroke susceptibility in several case–control association studies. However, the studies have reported apparently conflicting results, rendering precise assessment of the disease risk associated with the variants difficult. A meta-analysis was therefore conducted by including the studies that examined the association between two common polymorphisms (L55M and Q192R) in the coding region of *PON1* gene and the risk of stroke. Altogether 10 studies on L55M polymorphism and 11 studies on Q192R polymorphism were included in this meta-analysis. The results showed, although there was no significant association of the 55L allele with stroke [random effects OR = 1.09, 95% CI (0.93, 1.27), $P = 0.29$], the 192R allele conferred significant risk of stroke in the overall study population [random effects OR = 1.25, 95% CI (1.07, 1.46), $P = 0.006$]. Same pattern of results as both the allele contrasts was obtained for the homozygote contrasts and the dominant, recessive and additive models. Subgroup analyses for stroke type, age of patients and ethnicity revealed no association of the 55L allele with stroke, whereas the association of the 192R allele persisted significantly in the groups comprising ischemic stroke patients, stroke patients with mean age >60 years and Caucasian subjects. But no significant association of this allele with stroke susceptibility was detected in the East Asian population. Therefore, the results of this meta-analysis indicate, the Q192R polymorphism could be an important risk factor for stroke, especially in the Caucasian population.

Keywords Paraoxonase 1 · Polymorphism · Stroke · Association · Meta-analysis

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Introduction

Stroke is a heterogeneous multifactorial disorder that accounts for the largest number of mortality in the world after heart attack and cancer [1]. It is also the leading cause of disability in the world, placing heavy burden on the society [2]. Although stroke could be caused by any or a combination of the traditional risk factors such as hypertension, diabetes, smoking, hyperlipidemia etc., growing number of evidences suggest genetic factors play a crucial role in stroke etiology through their interactions with the environmental components [3]. It is, therefore, important to identify the gene variants contributing to stroke pathogenesis. The knowledge of the genetic factors influencing stroke susceptibility could be instrumental in enhancing the prediction of disease risk and devising efficient therapeutic strategies, based on targeted approach.

Several genetic association studies have accumulated evidence that a number of genes involved in cholesterol metabolism, inflammation, blood coagulation, homocysteine metabolism, rennin-angiotensin system etc. could be implicated in the development of stroke [4]. Of these genes, human serum paraoxonase 1 (*PON1*) stands as a potential candidate. PON1 is a calcium-dependent glycoprotein that is associated with high density lipoprotein (HDL) in serum [5]. It preserves HDL functions and prevents oxidation of the low density lipoproteins (LDL) by hydrolyzing lipid peroxides [6, 7]. It also metabolizes the

biologically active lipids in oxidized LDLs and thereby prevents the induction of monocyte-endothelial interactions on the arterial wall [8]. Therefore, the biological functions of PON1 suggest its role against the pathogenesis of atherosclerosis, the major underlying cause of ischemic stroke and other cardiovascular diseases. In addition, the findings that PON1 activity is lowered in vascular diseases and *PON1* knockout mice have increased susceptibility to atherosclerosis have further supported the role of PON1 as an antioxidant and antiatherogenic enzyme [6, 9, 10].

PON1 gene has two common single nucleotide polymorphisms (SNPs) in the coding region of the gene, leading to Gln(Q) → Arg(R) and Leu(L) → Met(M) substitutions at positions 192 and 55, respectively. These two variants have been shown to influence PON1 activity and serum concentrations independently [11, 12]. Several studies have shown both the Q192R (rs662) and L55M (rs854560) polymorphisms are associated with increased risk of coronary-artery disease (CAD), but there were inconsistencies in the results as other studies failed to detect any association [3]. Homozygosity for the 55L allele was shown to confer risk to CAD independently, but the subsequent studies demonstrated conflicting results [13]. Recent studies were also aimed to discern the role of these SNPs in stroke, following the common mechanistic links between stroke and CAD. But majority of the individual studies were of low statistical power, due to which the degree of association between the *PON1* gene variants and stroke could not be determined with reliability. Moreover, ethnic influences on studies in different populations across the world further obscured the true nature of association between these gene variants and stroke.

In order to investigate the precise relationship between the *PON1* gene variants and stroke, a study with high statistical power was necessary. Therefore, to quantify in a reliable manner the genetic risks associated with the *PON1* Q192R and L55M polymorphisms in stroke susceptibility, a meta-analysis of all available published studies was performed. In this meta-analysis, the overall association of the gene variants with stroke was investigated. To examine the possible influence of various factors like stroke-type, age and ethnicity on the overall results, subgroup analyses were also conducted. The subgroups were comprised of the studies on ischemic stroke, patients with mean age more than 60 years, East Asian and Caucasian populations.

Materials and methods

Study identification and selection of relevant studies

A literature search of the PubMed database was conducted to identify all articles that examined the association of the *PON1* gene variants with stroke. The terms "stroke", "paraoxonase1", "PON1" and "polymorphism" were used as search criteria. Articles reporting the association of the *PON1* Q192R and L55M polymorphisms with stroke were identified. All the studies that were published before June, 2008 were considered for initial screening. The articles written in English were considered only. The search results were limited to humans.

To assess the appropriateness of the studies for inclusion in this meta-analysis, the publications were read in their entirety. Abstracts, editorials and review articles were excluded. Clinically overt case–control studies included in this meta-analysis had to meet all of the following criteria: (a) stroke was diagnosed by neurologists as neurological deficits with characteristic symptoms, accompanied by abnormal brain lesions as evident in either magnetic resonance imaging (MRI) or computed tomography (CT), and patients with other cerebrovascular diseases were excluded, based on the reports of MRI or CT and other diagnostic tests, (b) control subjects were unrelated individuals, chosen randomly from the same geographic region, and without any symptomatic vascular disease as confirmed by physicians after physical examination and by evaluating clinical history and diagnostic tests, (c) cases with transient ischemic attack (TIA) or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) were excluded, and (d) genotype distributions in both cases and controls were available.

Data extraction

The following information were extracted from each study: name of the first author, year of publication, country, journal, racial descent of study population, demographics, number of cases and controls, genotyping methods, genotype and allele distributions and confirmation of diagnosis. If allele frequencies were not given, they were calculated from the corresponding genotype distributions.

Statistical analysis

For each variant of *PON1*, meta-analysis was performed to examine the overall association for the allele contrast, the contrast of homozygotes, and the recessive, dominant and additive models.

To measure the strength of genetic association for each gene variant, the odds ratios (ORs), together with the 95% confidence interval (CI) and the corresponding *P* value (the *P* value being significant if <0.05) were calculated. Heterogeneity between the studies was examined by Q-statistic, which is a weighted sum of squares of the deviations of individual study OR estimates from the overall estimate, [14]. Q follows a chi-square distribution with $r - 1$ (r is

the number of studies) degrees of freedom (*df*), when the ORs were homogeneous [15]. The heterogeneity was considered statistically significant with $P < 0.10$. Quantification of the heterogeneity was done with the I^2 metric ($I^2 = (Q - df)/Q$), which is independent of the number of studies in the meta-analysis [16]. The I^2 values falls within the range 0–100%, with higher values denoting greater degree of heterogeneity ($I^2 = 0$–25%, no heterogeneity; $I^2 = 25$–50%, moderate heterogeneity; $I^2 = 50$–75%, large heterogeneity; $I^2 = 75$–100%, extreme heterogeneity) [15]. The Mantel–Haenszel method was used to calculate the fixed-effect pooled ORs [17, 18]. The random-effects pooled ORs were calculated by the DerSimonian and Liard method [19]. As the studies are both clinically and methodologically diverse, heterogeneity between studies is an expected outcome [20]. If heterogeneity existed between studies, a pooled OR was estimated by the random-effects model, because this model assumes a genuine diversity in the results of the studies, incorporating the calculations of inter-study variability and provides wider CIs [15, 21]. In this article, the results from the random-effects model are reported only. To assess the publication bias for allele contrasts, the Egger regression test for funnel plot asymmetry [15, 22, 23] and the Begg–Mazumdar test, which is based on Kendall's tau [24], were carried out. To examine the effect of the studies with controls that are not in Hardy–Weinberg equilibrium, sensitivity analyses were performed by their exclusion [15]. Subgroup analyses of the studies on ischemic stroke, patients with mean age >60 years, East Asian and Caucasian populations, were also carried out. The statistical software StatsDirect (version 2.5.7) was used to analyze the data.

Results

Study inclusion

The initial search with the key words and subject terms identified a total of 22 studies. Of these, based on the searching criteria, thirteen articles were retrieved [25–37]. Due to unavailability of the article and the genotype data, two studies were excluded [36, 37]. Finally, eleven studies [25–35] were considered for the meta-analysis on the Q192R and L55M variants of the *PON1* gene. For subgroup analyses, studies were stratified according to the stroke type (ischemic stroke [25–31, 34, 35]), age (mean age of the patients >60 years [25–28, 30, 31, 33, 35]) and ethnicity (East Asian population [25, 29, 31, 33, 35] and Caucasian population [26–28, 30, 32]). One study investigated the association between the paraoxonase gene variants with different forms of stroke such as, small vessel disease, large vessel disease, cardioembolic stroke [28]. However, for each subtype of stroke, different control groups were included. To avoid complications for statistical analysis, only the data for the large vessel disease were considered for this meta-analysis. One study was conducted in a population of mixed ethnic background comprising subjects from Caucasian, African and Asian populations [34]. Studies with controls not in Hardy–Weinberg equilibrium (HWE) were also considered for the meta-analysis, but they were excluded in the sensitivity analysis.

In most of the studies included in this meta-analysis, stroke cases were incidental, not prevalent. Genotype determination in the included studies was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method, except for two studies [25, 32] where other genotyping techniques were used. To adjust the ORs for potential risk factors for vascular diseases such as hypertension, smoking habit, diabetes etc., logistic regression analysis was performed in some of the studies [26, 31, 32, 34].

Summary statistics

Characteristics of the included studies on *PON1* L55M and Q192R polymorphisms are shown in Table 1. The studies provided 1,484 cases and 4,075 controls for L55M and 1,726 cases and 4,379 controls for Q192R. The genotype distributions and the allele frequency of L55M and Q192R are shown in Tables 2 and 3, respectively. Out of the 10 studies on L55M included in this meta-analysis, the control group of one study did not follow HWE [30], and therefore, was excluded in the sensitivity analyses. For Q192R, there were altogether 11 studies included in this meta-analysis. In the sensitivity analyses for this gene variant, 3 studies were excluded where the control groups did not follow HWE [25, 33, 34].

Main results, sensitivity and subgroup analyses

In the meta-analysis of *PON1* L55M polymorphism (Table 4; Fig. 1), the summary ORs showed no statistically significant association of the L allele with the susceptibility to stroke as compared to the M allele under the random effects model, OR = 1.09 [95% CI (0.93, 1.27)]; $P = 0.29$. No inter-study heterogeneity was observed for this allelic variant ($I^2 = 20$; $P = 0.26$). In the sensitivity analysis, where the study with controls not in HWE was excluded, no heterogeneity was observed ($I^2 = 14.6$; $P = 0.31$), and no association of the L allele with stroke was found, the random effects OR = 1.05 [95% CI (0.90, 1.23)]; $P = 0.51$. The Begg-Mazumdar test, although indicated low power for this meta-analysis, showed no significant publication bias, Kendall's tau = 0.29; $P = 0.29$. The Egger's test also showed no publication

Table 1 Characteristics of the studies of *PON1* L55M and Q192R polymorphisms and stroke (*M/F* male/female)

Population	Stroke type	Case	Control	Polymorphisms	Reference
East Asian	Ischemic	350 patients (M/F = 1.3/1); mean age 67.4 ± 11.3 years	242 controls (M/F = 1/1); mean age 67.8 ± 8.4 years	L55M, Q192R	[25]
Caucasian	Ischemic	108 patients (77.8% male); mean age 64.2 ± 15.3 years	78 controls (57.1% male); mean age 63.2 ± 13.4 years	L55M, Q192R	[26]
Caucasian	Ischemic	126 patients (63.3% male); mean age 66.0 years	92 controls (60.7% male); mean age 65.0 years	L55M, Q192R	[27]
Caucasian	Ischemic	136 patients (M/F = 89/47); mean age 65.8 ± 11.5 years	238 controls (M/F = 142/96); mean age 66.0 ± 11.0 years	L55M, Q192R	[28]
East Asian	Ischemic	153 patients (M/F = 92/61); mean age 59.8 ± 12.0 years	153 controls (M/F = 89/64); mean age 57.7 ± 8.8 years	L55M, Q192R	[29]
Caucasian	Ischemic	65 patients (46.2% male); mean age 70.4 ± 11.9 years	84 controls (31% male); mean age 67.7 ± 3.9 years	L55M, Q192R	[30]
East Asian	Ischemic	242 patients (54.5% male); mean age 70.7 ± 12.0 years	336 controls (45.2% male); mean age 71.0 ± 5.9 years	Q192R	[31]
Caucasian	Ischemic and hemorrhagic	81 patients (M/F = 69/12); mean age 58.0 ± 9.3 years	2,553 controls (M/F = 2,216/337); mean age 64.0 ± 7.5 years	L55M, Q192R	[32]
East Asian	Cerebral infarction	112 patients (M/F = 69/46); mean age 70.0 ± 9.9 years	106 controls (M/F = 48/58); mean age 69.0 ± 9.6 years	L55M, Q192R	[33]
Mixed (Caucasian, African and Asian)	Ischemic	118 patients (45.8% male); mean age 36.3 ± 6.6 years	118 controls (45.8% male); mean age 36.6 ± 6.8 years	L55M, Q192R	[34]
East Asian	Ischemic	235 patients (M/F = 115/120); mean age 69.5 ± 8.5 years	431 controls (M/F = 321/110); mean age 63.6 ± 9.1 years	L55M, Q192R	[35]

bias, $P = 0.87$. In the genotype contrasts LL vs. MM, LL vs. LM vs. MM, LL vs. (LM + MM), no statistically significant association was detected, although some OR values indicated high risk for stroke. The carriers of L allele (genotypes LL + LM) showed significant association as compared to the MM homozygotes, the random effects OR = 1.64 [95% CI (1.12, 2.41)]; $P = \mathbf{0.01}$. However, in the sensitivity analysis this association became non-significant, the random effects OR = 1.39 [95% CI (0.97, 1.99)]; $P = 0.07$.

The results on the Q192R polymorphism (Table 4 and Fig. 2) indicated, the R allele could contribute significantly to stroke susceptibility as compared to the Q allele, the random effects OR = 1.25 [95% CI (1.07, 1.46)]; $P = \mathbf{0.006}$. No significant publication bias was found by the Begg-Mazumdar test, Kendall's tau = −0.05; $P = 0.76$. The Egger's test also showed no significant publication bias $P = 0.95$. However, large heterogeneity among the studies was observed ($I^2 = 59.3$; $P = \mathbf{0.006}$). The degree of heterogeneity was not altered much in the sensitivity analysis ($I^2 = 60.2$; $P = \mathbf{0.01}$). In the genotype contrasts RR vs. QQ, RR vs. RQ vs. QQ, RR vs. (RQ + QQ) and (RR + RQ) vs. QQ, statistically significant associations were detected in all studies and in sensitivity analyses, indicating both the RR genotype and the R allele carriers are at high risk for stroke.

In the subgroup analyses (Table 5), no association between the L55M polymorphism and stroke was revealed in any of the groups analyzed. However, the Q192R polymorphism was found to be significantly associated with stroke susceptibility in the groups comprising Caucasian subjects, the random effects model, OR = 1.32 [95% CI (1.01, 1.71)]; $P = \mathbf{0.04}$, ischemic stroke patients, the random effects model, OR = 1.21 [95% CI (1.02, 1.43)]; $P = \mathbf{0.03}$, and the patients with mean age >60 years, the random effects model, OR = 1.20 [95% CI (1.03, 1.41)]; $P = \mathbf{0.02}$. Moderate to large heterogeneity persisted in all the groups for the Q192R polymorphism. Interestingly, no significant association between the R allele and stroke was detected in the East Asian population, the random effects model, OR = 1.14 [95% CI (0.92, 1.42)]; $P = 0.23$.

Discussion

The first report evaluating the role of *PON1* polymorphisms in the predisposition of ischemic stroke was produced by Imai et al. [35], where the R allele of Q192R polymorphism was found to be an independent risk factor for the disease in a Japanese population. The same study also investigated the relationship between the L55M polymorphism and ischemic stroke, but could not detect any association. Previous studies demonstrated the L55M and Q192R polymorphisms in the *PON1* gene could contribute to the risk of CAD, possibly through

Table 2 The distribution of the *PON1* L55M genotypes and the allele frequency for stroke patients and controls (values in parentheses are the corresponding percentages)

First author, year	Reference	Population	Distribution of PON1 genotypes						Frequency of PON1 alleles			
			LL		LM		MM		L		M	
			Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Shin, 2008	[25]	East Asian	317 (91)	215 (89)	33 (9)	27 (11)	0 (0)	0 (0)	667 (95)	457 (94)	33 (5)	27 (6)
Can Demirdogen, 2008	[26]	Caucasian	54 (50)	34 (44)	41 (38)	30 (38)	13 (12)	14 (18)	149 (69)	98 (63)	67 (31)	58 (37)
Schiavon, 2007	[27]	Caucasian	55 (44)	43 (47)	61 (48)	39 (42)	10 (8)	10 (11)	171 (68)	125 (68)	81 (32)	59 (32)
Slowik, 2007	[28]	Caucasian	55 (40)	96 (41)	66 (49)	114 (48)	15 (11)	26 (11)	176 (65)	306 (65)	96 (35)	166 (35)
Huang, 2006	[29]	East Asian	148 (97)	143 (93)	5 (3)	10 (7)	0 (0)	0 (0)	301 (98)	296 (97)	5 (2)	10 (3)
Aydin, 2006	[30]	Caucasian	12 (18)	20 (24)	42 (65)	29 (34)	11 (17)	35 (42)	66 (51)	69 (41)	64 (49)	99 (59)
Ranade, 2005	[32]	Caucasian	31 (38)	1,015 (40)	45 (56)	1,199 (47)	5 (6)	321 (13)	107 (66)	3,229 (64)	55 (34)	1,841 (36)
Ueno, 2003	[33]	East Asian	93 (83)	98 (92)	16 (14)	8 (8)	3 (3)	0 (0)	202 (90)	204 (96)	22 (10)	8 (4)
Voetsch, 2002	[34]	Mixed (Caucasian, African and Asian)	53 (45)	56 (47)	55 (47)	48 (41)	10 (8)	14 (12)	161 (68)	160 (68)	75 (32)	76 (32)
Imai, 2000	[35]	East Asian	203 (86)	371 (86)	31 (13)	55 (13)	1 (0)	5 (1)	437 (93)	797 (92)	33 (7)	65 (8)

Table 3 The distribution of the *PON1* Q192R genotypes and the allele frequency for stroke patients and controls (values in parentheses are the corresponding percentages)

First author, year	Reference	Population	Distribution of PON1 genotypes						Frequency of PON1 alleles			
			QQ		QR		RR		Q		R	
			Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Shin, 2008	[25]	East Asian	156 (45)	103 (43)	194 (55)	139 (57)	0 (0)	0 (0)	506 (72)	345 (71)	194 (28)	139 (29)
Can Demirdogen, 2008	[26]	Caucasian	48 (44)	31 (40)	42 (39)	40 (51)	18 (17)	7 (9)	138 (64)	102 (65)	78 (36)	54 (35)
Schiavon, 2007	[27]	Caucasian	58 (46)	44 (48)	56 (44)	36 (39)	12 (10)	12 (13)	172 (68)	124 (67)	80 (32)	60 (33)
Slowik, 2007	[28]	Caucasian	75 (55)	135 (57)	46 (34)	88 (37)	15 (11)	15 (6)	196 (72)	358 (75)	76 (28)	118 (25)
Huang, 2006	[29]	East Asian	21 (14)	15 (10)	79 (52)	82 (54)	53 (35)	56 (37)	121 (40)	112 (37)	185 (60)	194 (63)
Aydin, 2006	[30]	Caucasian	14 (22)	38 (45)	40 (62)	35 (42)	11 (17)	11 (13)	68 (52)	111 (66)	62 (48)	57 (34)
Baum, 2006	[31]	East Asian	32 (13)	65 (21)	119 (49)	135 (44)	91 (38)	110 (35)	183 (38)	265 (43)	301 (62)	355 (57)
Ranade, 2005	[32]	Caucasian	25 (31)	1,328 (53)	45 (56)	985 (39)	11 (14)	214 (8)	95 (59)	3,641 (72)	67 (41)	1,413 (28)
Ueno, 2003	[33]	East Asian	22 (20)	24 (23)	40 (36)	37 (35)	50 (45)	45 (42)	84 (38)	85 (40)	140 (62)	127 (60)
Voetsch, 2002	[34]	Mixed (Caucasian, African and Asian)	36 (31)	50 (42)	63 (53)	62 (53)	19 (16)	6 (5)	135 (57)	162 (69)	101 (43)	74 (31)
Imai, 2000	[35]	East Asian	11 (5)	59 (14)	95 (40)	182 (42)	129 (55)	190 (44)	117 (25)	300 (35)	353 (75)	562 (65)

Table 4 Summary of odds ratios (OR) with confidence intervals (CI) for various genetic contrasts of the *PON1* L55M and Q192R polymorphisms for stroke patients

Genetic contrasts	Populations	Studies (n)	Alleles/ Genotypes (n)	Random effects [OR (95% CI)]	Random effects *P* value	I^2 (%)	Q test *P* value
L versus M	All	10	11,118	1.09 (0.93, 1.27)	0.29	20.0	0.26
	All in HWE	9	10,820	1.05 (0.90, 1.23)	0.51	14.6	0.31
R versus Q	All	11	12,210	1.25 (1.07, 1.46)	**0.006**	59.3	**0.006**
	All in HWE	8	10,118	1.28 (1.06, 1.54)	**0.01**	60.2	**0.01**
LL versus MM	All	8	2,782	1.40 (0.98, 1.99)	0.06	0	0.69
	All in HWE	7	2,704	1.34 (0.92, 1.95)	0.13	0	0.64
RR versus QQ	All	10	3,206	1.78 (1.23, 2.57)	**0.002**	55.5	**0.02**
	All in HWE	8	2,954	1.72 (1.14, 2.58)	**0.01**	56.2	**0.02**
LL versus LM versus MM	All	10	5,559	1.09 (0.90, 1.33)	0.37	0	0.74
	All in HWE	9	5,410	1.06 (0.87, 1.30)	0.56	0	0.75
RR versus RQ versus QQ	All	11	6,105	1.25 (1.07, 1.46)	**0.005**	18	0.27
	All in HWE	8	5,059	1.29 (1.07, 1.55)	**0.007**	20.2	0.27
LL versus (LM + MM)	All	10	5,559	0.97 (0.82, 1.16)	0.78	0	0.52
	All in HWE	9	5,410	0.99 (0.83, 1.18)	0.91	0	0.47
RR versus (RQ + QQ)	All	10	5,513	1.32 (1.06, 1.66)	**0.01**	32.1	0.15
	All in HWE	8	5,059	1.28 (1.04, 1.59)	**0.02**	18.0	0.29
(LL + LM) versus MM	All	8	4,661	1.64 (1.12, 2.41)	**0.01**	22.7	0.25
	All in HWE	7	4,512	1.39 (0.97, 1.99)	0.07	0	0.59
(RR + RQ) versus QQ	All	11	6,105	1.40 (1.05, 1.87)	**0.02**	69.4	**0.0003**
	All in HWE	8	5,059	1.50 (1.03, 2.19)	**0.04**	73.1	**0.0005**

HWE Hardy–Weinberg equilibrium

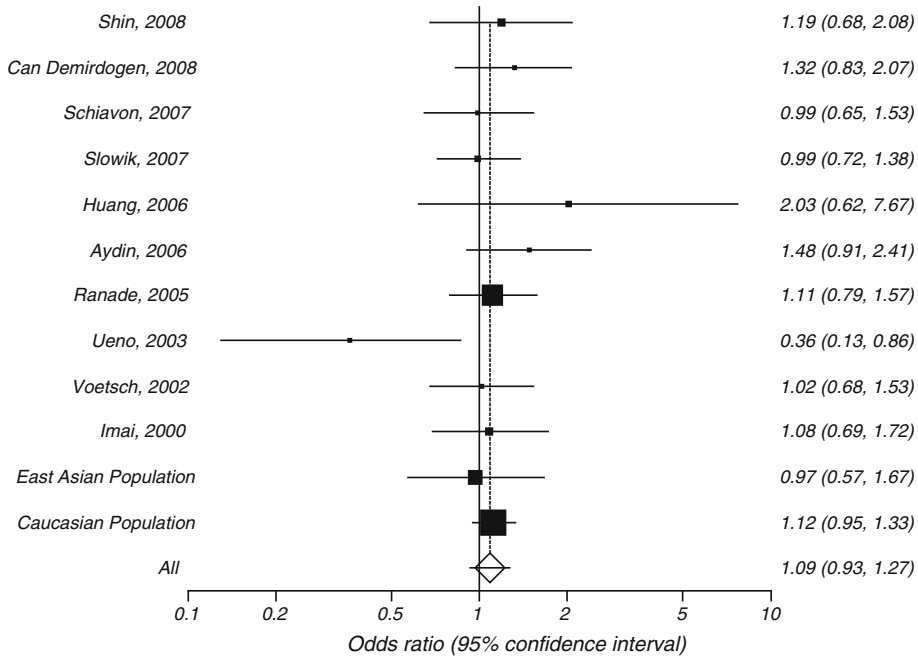

Fig. 1 Results of the published studies of the association between *PON1* L55M polymorphism and stroke in the East Asian, Caucasian and combined populations. Each study is shown by an OR estimating the outcome of the comparison of the L allele against the M allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

exaggeration of atherosclerotic events [12, 38–40]. However, discordant results were also reported from studies in different populations [41–44]. A meta-analysis on Q192R polymorphism failed to show any strong evidence that the polymorphism is associated with CAD risk in either Caucasian men or women [45]. Another meta-analysis, with higher number of studies included, demonstrated there was no overall association of CAD with the L55M

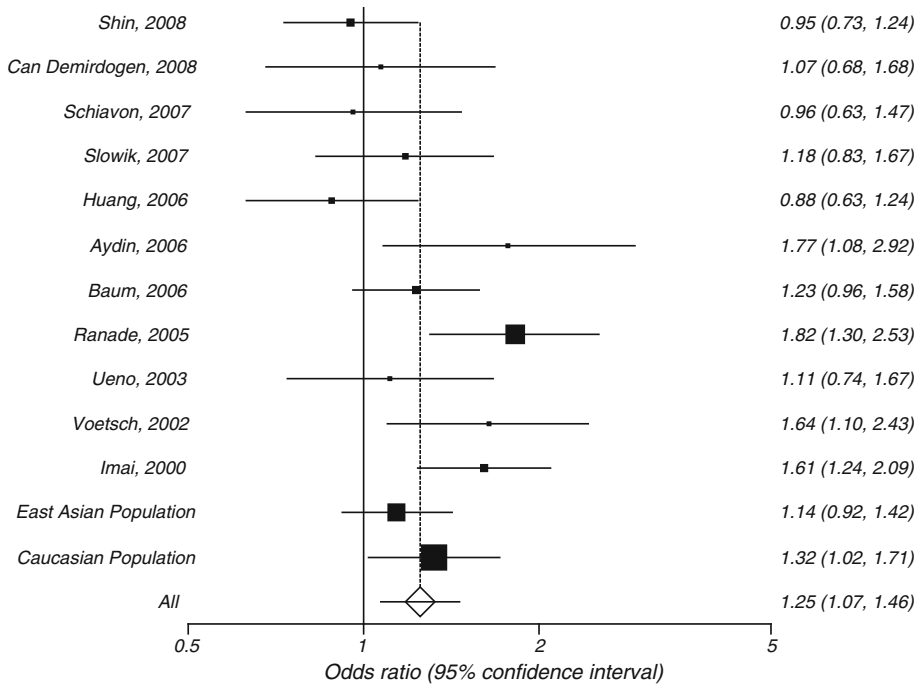

Fig. 2 Results of the published studies of the association between *PON1* Q192R polymorphism and stroke in the East Asian, Caucasian and combined populations. Each study is shown by an OR estimating the outcome of the comparison of the R allele against the Q allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

Table 5 Subgroup analyses for allele contrasts of the *PON1* L55M and Q192R polymorphisms for stroke patients

Subgroups	Allelic contrasts	Studies (n)	Alleles (n)	Random effects [OR (95% CI)]	Random effects *P* value	I^2 (%)	Q test *P* value
East Asian population	L vs. M	4	3,564	0.97 (0.57, 1.67)	0.93	62.5	**0.04**
	R vs. Q	5	4,668	1.14 (0.92, 1.42)	0.23	65.9	**0.02**
Caucasian population	L vs. M	5	7,082	1.12 (0.95, 1.33)	0.17	0	0.6
	R vs. Q	5	7,070	1.32 (1.01, 1.71)	**0.04**	56.1	0.06
Ischemic stroke	L vs. M	8	5,450	1.12 (0.96, 1.31)	0.13	0	0.7
	R vs. Q	9	6,558	1.21 (1.02, 1.43)	**0.03**	56.8	**0.02**
Mean age of patients >60 years	L vs. M	7	4,802	1.07 (0.86, 1.33)	0.54	39.1	0.13
	R vs. Q	8	5,910	1.20 (1.03, 1.41)	**0.02**	44.6	0.08

polymorphism, whereas weak association was detected with the Q192R polymorphism, which was of uncertain relevance as there was no significant association among the larger studies [46]. Since there may be common risk traits for both CAD and stroke, studies were also initiated to test the associations between paraoxonase gene polymorphisms and stroke, assuming they could lead to both the disorders through their participation in systemic atherogenesis [35, 47]. A number of studies investigating the role of *PON1* polymorphisms in stroke susceptibility followed the study of Imai et al. But the studies yielded apparently conflicting results.

There could be several factors contributing to discordant findings among individual studies. Small sample size is one of them, which often enhances the chance factor for false-positive or false-negative findings. In meta-analysis, however, the false-positive and false-negative results neutralize each others as large number of studies is pooled, and the increase of overall statistical power leads to a more precise and accurate measure of association. Variation among results of different studies might also be the consequence of different sampling strategies and/or ethnical variation among the study populations. If considerable number of reports are available on ethnically distinct populations, separate meta-analysis on each of the major population groups might provide significant insight on the cause of discordant results.

To help clarify the evidences from different studies, a meta-analysis was performed on the data pooled from the available published studies examining the association between *PON1* polymorphisms and stroke. The relationship between stroke and the L55M and Q192R polymorphisms in the coding region of *PON1* gene was investigated. The overall results suggest, although the 55L

allele did not show any association with stroke, the 192R allele could confer risk to stroke susceptibility significantly. The results remained unaffected in the sensitivity analysis where studies with controls not in Hardy–Weinberg equilibrium were excluded, indicating reliability and stability of the results. Subgroup analyses taking into account patient characteristics and ethnicity failed to identify any association between the 55L allele and stroke susceptibility, but significant association of the 192R allele with stroke risk was revealed in the groups comprising the patients with ischemic stroke, the patients with mean age more than 60 years and the subjects from the Caucasian population, but not from the East Asian population.

In the retrospective cumulative meta-analysis of the studies on L55M polymorphism (data not shown), the random effects OR of the allelic contrast was found to decrease from 1.08 (first study in 2000) to 0.83 (three studies in 2003). After that the OR showed an increasing trend: 1.07 (six studies in 2006) to 1.09 (ten studies in 2008), even though the association remained non-significant throughout. However, the random effects OR for the Q192R polymorphism showed a decreasing trend from 1.61 (first study in 2000) through 1.47 (three studies in 2003) and 1.38 (seven studies in 2006) to 1.25 (eleven studies in 2008), despite the fact that the association remained statistically significant each time.

As the publication of findings often depends on the expectation of researchers, false-negative results may be suppressed or false-positive results magnified [48, 49]. The results of this study, however, did not show any significant publication bias. However, since the number of studies included in this meta-analysis was small and large inter-study heterogeneity was observed, these results should be considered with caution. The possibility that the results were affected by false-positive or false-negative findings could not be ruled out. In this meta-analysis, although there was no heterogeneity among the studies on the L55M polymorphism, large heterogeneity was observed among the studies on the Q192R polymorphism, which did not alter much in the sensitivity analysis. The observed heterogeneity could be attributable to differences in several factors such as ethnic variations, environmental factors and methodological factors in design and conduct of the studies. Among these factors, ethnicity could play a crucial role as the allele frequencies of the L55M and Q192R polymorphisms were found to vary between the East Asian and the Caucasian populations: the frequency of the L allele in the East Asian population was 94% in controls and 95% in cases, whereas, in the Caucasian population it was 63% in controls and 65% in cases. The R allele frequency in the East Asian population was 55% in controls and 54% in cases, whereas in the Caucasians, the frequency was 24% in controls and 35% in cases. An earlier study also revealed very distinctive distribution of the allele frequencies of the 192R and the 55M alleles for the Caucasian, Asian and Afro-American populations [50]. In this study, the observed positive association of the 192R allele with stroke susceptibility in the Caucasian population, and the lack of association in the East Asian population suggest that ethnicity could be a potential determinant of the disease risk, owing to the differences in genetic architecture of these two populations.

It is also important to note that a linkage disequilibrium (LD) exists between the 192R and the 55L alleles [51]. A study in the Caucasian population found, nearly all of the 192R alleles encode 55L and the 192R/55M genotype was very rare [52]. However, in a population of African descent, this LD appeared to be less extreme [51]. A comparative study revealed, the Euro-Brazilians with 192RR genotype lacked the 55M allele, whereas the same was present in a group of Afro-Brazilians [50]. The high LD, observed in this study, in the Caucasian and in the Asian populations was mainly due to the absence or very low frequency of the MR haplotype [50]. The LD existing between these two polymorphisms may be in part responsible for the previously reported relationship between the 55L variant and the risk of stroke, which is not evident in this study.

This study has some limitations that need to be acknowledged. First, the number of available studies that could be included in this meta-analysis was moderate. Therefore, the results could be influenced by the factors like random error, publication bias etc. Second, the case–control association studies included in this meta-analysis were small or medium-sized, with little statistical power to reliably assess the relevance of the SNPs to the risk of stroke. Third, this study could not address gene–gene and gene-environment interactions. Fourth, variation in clinical classification of stroke patients and controls, genotyping and ethnic polymorphy among the included studies could influence the overall outcome of this meta-analysis. Fifth, since the number of studies included in the subgroup analyses was small, the results, although indicative, lack sufficient reliability to confirm or refute an association in a definitive manner.

In conclusion, the results of this meta-analysis suggest the *PON1* L55M polymorphism is not associated with the susceptibility to stroke in the study populations. However, the Q192R polymorphism of the same gene could be regarded as a potential risk factor for stroke, especially in the Caucasian population.

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