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# Association between paraoxonase gene and stroke in the Han Chinese population

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## Abstract

**Background:** The human paraoxonase (*PON*) gene family has three isoforms: *PON1*, *PON2* and *PON3*. These genes are implicated as potential risk factors of cerebrovascular disease and can prevent oxidative modification of low-density lipoproteins and atherosclerosis. This study evaluated the association between the genetic variants of all three *PON* genes and the risks of total stroke, ischemic stroke and hemorrhagic stroke in the Han Chinese population.

**Methods:** A total of 1016 subjects were recruited, including 508 healthy controls and 498 patients (328 with ischemic stroke and 170 with hemorrhagic stroke). A total of 11 single nucleotide polymorphisms (SNPs) covering the *PON* genes were genotyped for statistical analysis. Two of the 11 SNPs (rs662 and rs854560) were contextualized in a meta-analysis of ischemic stroke.

**Results:** The presence of rs705381 (−162) in the promoter region of *PON1* was significantly associated with total stroke ( $P_{adjusted} = 0.0007$ ,  $OR = 0.57$  [95% CI = 0.41-0.79]) and ischemic stroke ( $P_{adjusted} = 0.0017$ ,  $OR = 0.54$  [95% CI = 0.37-0.79]) when analyzed using a dominant model, but was not associated with hemorrhagic stroke. There was also a nominal association between rs854571 (−824) and total stroke. Meta-analysis demonstrated a significant nominal association between rs662 and ischemic stroke, but there was no evidence of an association between rs662 and ischemic stroke risk in a single site association study.

**Conclusions:** These findings indicate that polymorphisms of *PON1* gene may be a risk factor of stroke.

**Keywords:** Polymorphisms, Paraoxonase gene, Hemorrhagic stroke, Ischemic stroke, Association

## Background

Stroke is recognized as one of the leading causes of death and severe neurological disability worldwide. Ischemic and hemorrhagic stroke are the two primary subtypes [1]. Data from family-based studies [2], twin studies [3,4], and animal experiments [5,6] indicate that genetic factors play a major role in stroke. A small isolated group of strokes have previously been ascribed to single-gene disorders [7].

Intermediate phenotypes of stroke are seen clinically. Atherosclerosis, as an intermediate phenotype of stroke, has been extensively investigated as a major underlying cause of cardio- and cerebrovascular disease [8-10]. There is also a strong inverse association between high-density lipoprotein (HDL) levels and the development of atherosclerosis, and similar results have been found between low-density lipoprotein (LDL) peroxidation and the development of atherosclerosis [11,12].

The paraoxonase (*PON*) gene family comprises three isoforms, *PON1*, *PON2* and *PON3*, located in 7q21.3-22.1 [13]. The 60 to 80% structural similarity among these three members accounts for their functional similarity [13,14]. All three isoforms have been implicated as candidate genes for atherosclerosis and cardiovascular diseases due to their ability to attenuate lipid peroxidation, and due to their antioxidant and antiatherogenic effects [15-17].

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Low levels of PON activity are thought to increase the risk of atherosclerosis [18], and thereby contribute to a predisposition towards stroke, coronary artery disease (CAD) and vascular disorders in diabetes [19-21]. Other studies have demonstrated a positive association between single nucleotide polymorphisms (SNPs) in *PON* genes and stroke susceptibility [22-25], although conflicting results have been seen in different ethnic groups [26-28]. However, there are limited number of prospective studies validating the association between *PON* genes and the risk of stroke in the Han Chinese population [26,28-30].

A negative association has previously been demonstrated between SNPs in the coding region of *PONI* and *PON2*, and the development of stroke. In this study we wanted to evaluate the levels of ischemic and hemorrhagic risk conferred by SNPs in the whole *PON* family in a large Chinese population. With this aim in mind, we conducted a case-control study in the Han Chinese population to evaluate the possible association of *PON* family genes with total stroke and its subtypes.

## Methods

### Subjects

The study sample included 508 healthy controls and 498 patients, including 328 with ischemic stroke and 170 with hemorrhagic stroke who presented consecutively to the Department of Neurology, Beijing Tiantan Hospital, between December 2010 and March 2011. The subjects were unrelated to one another and were recruited from the Han Chinese population.

Hemorrhagic stroke included hypertensive cerebral hemorrhage and subarachnoid hemorrhage. Patients with hemorrhage due to trauma, tumor, vascular malformation and coagulopathy were excluded. Ischemic stroke was defined as a sudden onset of focal or global neurologic deficit with signs and symptoms persisting for more than 24 h. Patients with a history or occurrence of transient ischemic attack, cerebral embolism, cerebral trauma, cerebrovascular malformations, coagulation disorders, autoimmune diseases, tumors, peripheral vascular disease, or chronic infection diseases were excluded from the study.

All diagnoses were confirmed by brain computed tomography and/or magnetic resonance imaging. The brain images were independently assessed by a technologist and a physician.

Control subjects were recruited from the health examination department of the Beijing Tiantan Hospital. These subjects had no clinical or radiological evidence of stroke and other neurological diseases. They were also free from autoimmune disease, liver disease, nephrosis, and hematological disorders

Sex, age, total plasma cholesterol (TC), triglycerides (TG), HDL, and LDL cholesterol were documented on entry into the study. Potential vascular risk factors were

evaluated, including hypertension, diabetes mellitus, atrial fibrillation, and ischemic heart disease. Hypertension was defined according to WHO/ISH criteria [31] as systolic blood pressure  $\geq 140$  mmHg and/or diastolic pressure  $\geq 90$  mmHg with concomitant use of antihypertensive medications. Diabetes mellitus was defined as fasting plasma glucose  $\geq 7.0$  mmol/L or current treatment with anti-diabetic drugs.

The experimental protocol was approved by the Ethics Committee of the Beijing Tiantan Hospital. Written informed consent was obtained from all participants prior to entering the study.

### Genotyping

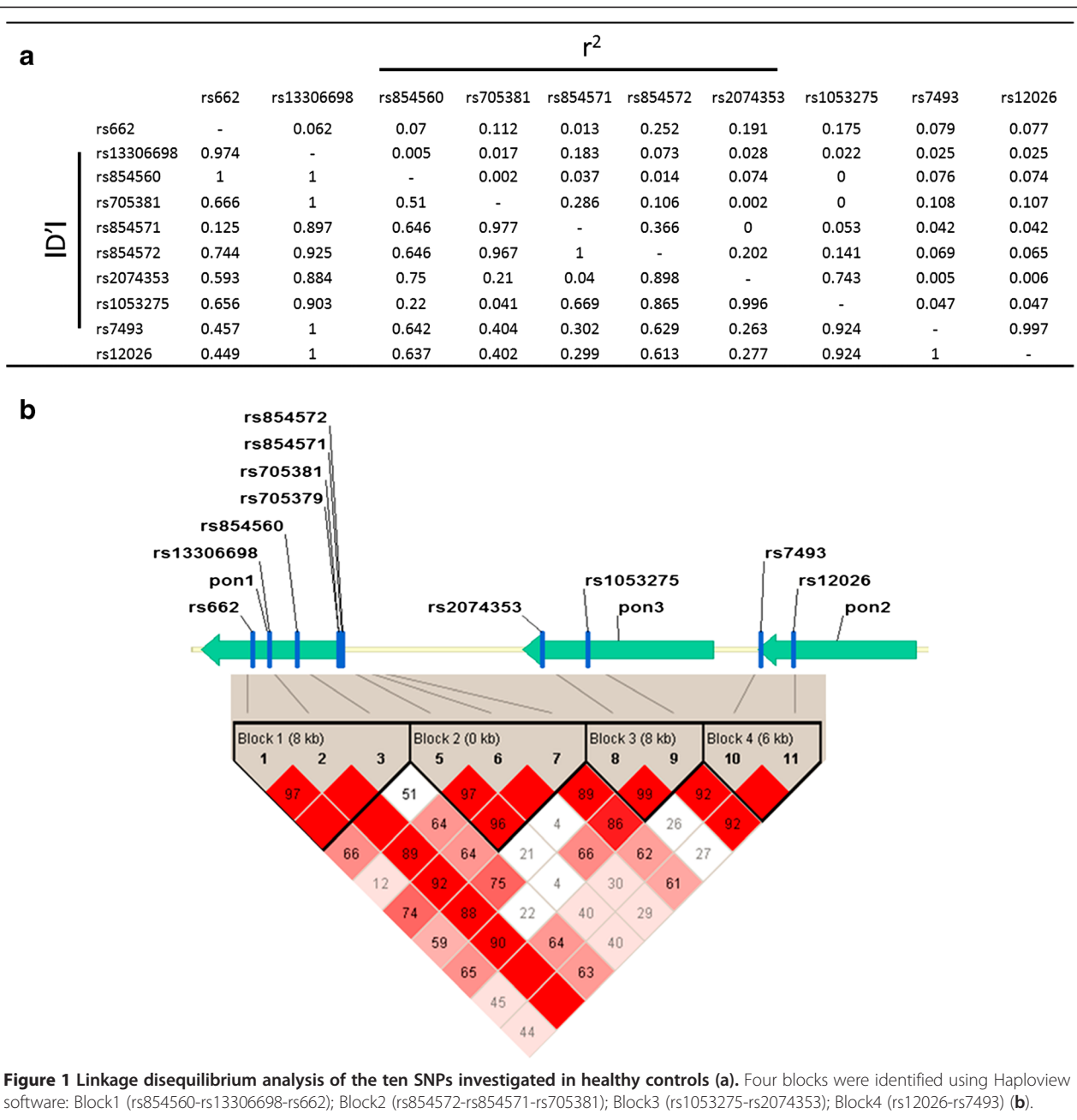
Eleven single nucleotide polymorphisms (SNPs) were genotyped. These included: rs662 (Gln192Arg), rs13306698 (Arg160Gly), rs854560 (Leu55Met) in coding region of *PONI*; rs705379 (-107/-108), rs705381 (-160/-162), rs854571 (-824/-832), rs854572 (-907/-909) in the promoter of *PONI*; rs12026 (Ala148Gly) and rs7493 (Ser311Cys) of *PON2*, together with rs2074353 (located in intron) and rs1053275 (Ala99Ala) for *PON3*.

The SNPs were genotyped using the Sequenom Mass ARRAY platform (Sequenom, San Diego, CA) according to the iPLEX Gold Application Guide available at (<http://www.sequenom.com/sites/genetic-analysis/applications/snp-genotyping>). The genotyping analysis was undertaken according to the manufacturer's protocol, using recommended reagents in the iPLEX Gold SNP genotyping kit. Briefly, specific assays were designed using the Mass ARRAY Assay Design software package (v3.1). The process involved a locus-specific PCR reaction based on a locus-specific primer extension reaction. Residual nucleotides were dephosphorylated with SAP enzymes before undertaking the iPLEX GOLD primer extension reactions.

**Table 1 Comparison of clinical variables between total strokes and control subjects**

Variables	Stroke cases (n = 498)	Control cases (n = 498)
Ischemic stroke, n	328	
Hemorrhagic stroke, n	170	
Age, years	60.45 $\pm$ 14.27*	56.48 $\pm$ 4.55
Male, n (%)	142 (28)	140 (28)
TC, mmol/L	4.41 $\pm$ 1.31	4.36 $\pm$ 1.33
TG, mmol/L	1.54 $\pm$ 0.95	1.56 $\pm$ 1.26
HDL, mmol/L	1.10 $\pm$ 0.28*	1.28 $\pm$ 0.27
LDL, mmol/L	2.54 $\pm$ 0.89	2.52 $\pm$ 0.56
Hypertension, n (%)	413 (83)*	310 (62)
Diabetes, n (%)	130 (26)	122 (24)

Data are shown as mean  $\pm$  standard deviation (SD) or as n (%). Abbreviations: TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.\*Significant differences between cases and controls.



Following the single-base extension reactions the products were desalinated with Spectro CLEAN resin (Sequenom). A 10 nL aliquot of the desalinated product was spotted onto a 384-format Spectro CHIP with the Mass ARRAY Nanodispenser. Mass determination was carried out with the MALDI-TOF mass spectrometer and Mass ARRAY Type 4.0 software was used for data acquisition.

SNP genotypes were named using cluster analysis with a default parameter setting. Genotypes were further reviewed manually to correct classification errors caused by clustering artifacts.

### Statistical analysis

Statistical analysis was undertaken using PLINK software (<http://pngu.mgh.harvard.edu/~purcell/plink/>) [32]. Hardy-Weinberg equilibrium tests (HWE) were performed for each SNP, and association tests were undertaken using additive, dominant, or recessive genetic models.

Logistic regression was used for risk stratification with or without covariate adjustments determined by significant differences between total stroke patients and controls (i.e. age, HDL, and hypertension). The model with the highest likelihood was considered to provide the best-fit genetic model for each SNP. Haplotype-based

**Table 2 Association between SNPs and total stroke using the additive, dominant, genotype, and the recessive models**

SNP	Model	Allele or geno	F_Stroke	F_Control	T-Statistic		Logistic Regression	
					OR (95% CI)	<i>P</i> <sub>unadjusted</sub>	OR (95% CI)	<i>P</i> <sub>adjusted</sub>
rs854571	Additive	C > T	298/992	344/982	0.79(0.65-0.96)	<b>1.71E-02</b>	0.80(0.65-0.98)	<b>3.30E-02</b>
	Dominant	CC + CT/TT	253/496	289/491	0.73(0.57-0.94)	<b>1.33E-02</b>	0.75(0.57-0.99)	<b>3.96E-02</b>
	Recessive	CC/CT + TT	45/496	55/491	0.79(0.52-1.20)	2.69E-01	0.75(0.48-1.17)	2.06E-01
rs13306698	Additive	A > G	98/1016	98/988	0.97(0.71-1.31)	8.31E-01	1.00(0.71-1.40)	9.99E-01
	Dominant	AA + AG/GG	97/508	96/494	0.98(0.71-1.34)	8.92E-01	1.02(0.72-1.44)	9.20E-01
	Recessive	AA/AG + GG	1/508	2/494	0.49(0.04-5.37)	5.55E-01	0.39(0.03-5.06)	4.74E-01
rs854572	Additive	C > G	443/1004	413/964	1.05(0.88-1.26)	5.69E-01	1.09(0.89-1.32)	4.08E-01
	Dominant	CC + CG/GG	343/502	324/482	1.05(0.81-1.38)	7.10E-01	1.11(0.82-1.48)	5.04E-01
	Recessive	CC/CG + GG	100/502	89/482	1.10(0.80-1.51)	5.62E-01	1.13(0.80-1.61)	4.93E-01
rs7493	Additive	C > G	192/1016	176/974	1.06(0.84-1.33)	6.32E-01	1.00(0.78-1.29)	9.89E-01
	Dominant	CC + CG/GG	173/508	163/487	1.03(0.79-1.34)	8.45E-01	1.00(0.75-1.34)	9.85E-01
	Recessive	CC/CG + GG	19/508	13/487	1.42(0.69-2.90)	3.41E-01	1.00(0.46-2.18)	9.93E-01
rs662	Additive	G > A	389/1014	356/978	1.08(0.91-1.29)	3.86E-01	1.05(0.87-1.28)	5.93E-01
	Dominant	GG + GA/AA	303/507	282/489	1.09(0.85-1.40)	5.02E-01	1.05(0.80-1.39)	7.31E-01
	Recessive	GG/GA + AA	86/507	74/489	1.15(0.82-1.61)	4.32E-01	1.12(0.77-1.62)	5.65E-01
rs12026	Additive	C > G	192/1010	174/978	1.09(0.86-1.37)	4.80E-01	1.05(0.81-1.35)	7.17E-01
	Dominant	CC + CG/GG	173/505	162/489	1.05(0.81-1.37)	7.07E-01	1.05(0.78-1.40)	7.56E-01
	Recessive	CC/CG + GG	19/505	12/489	1.55(0.75-3.24)	2.39E-01	1.13(0.51-2.50)	7.72E-01
rs1053275	Additive	A > G	203/1000	186/994	1.10(0.89-1.37)	3.82E-01	1.10(0.86-1.39)	4.53E-01
	Dominant	AA + AG/GG	179/500	165/497	1.12(0.86-1.46)	3.88E-01	1.13(0.85-1.51)	3.90E-01
	Recessive	AA/AG + GG	24/500	21/497	1.14(0.63-2.08)	6.62E-01	1.04(0.54-1.99)	9.17E-01
rs705381	Additive	G > A	106/988	151/990	0.67(0.51-0.87)	<b>3.13E-03*</b>	0.67(0.50-0.89)	<b>5.80E-03*</b>
	Dominant	GG + GA/AA	95/494	144/495	0.58(0.43-0.78)	<b>3.20E-04*</b>	0.57(0.41-0.79)	<b>7.10E-04*</b>
	Recessive	GG/GA + AA	11/494	7/495	1.59(0.61-4.13)	3.43E-01	1.64(0.58-4.61)	3.52E-01
rs2074353	Additive	A > G	253/996	230/982	1.11(0.91-1.36)	3.16E-01	1.12(0.90-1.40)	3.09E-01
	Dominant	AA + AG/GG	219/498	197/491	1.17(0.91-1.51)	2.20E-01	1.23(0.93-1.62)	1.46E-01
	Recessive	AA/AG + GG	34/498	33/491	1.02(0.62-1.67)	9.47E-01	0.91(0.53-1.57)	7.31E-01
rs854560	Additive	A > T	41/1014	39/996	1.03(0.66-1.61)	8.84E-01	0.95(0.58-1.56)	8.37E-01
	Dominant	AA + AT/TT	40/507	38/498	1.04(0.65-1.65)	8.78E-01	0.97(0.58-1.62)	9.12E-01
	Recessive	AA/AT + TT	1/507	1/498	0.98(0.06-15.75)	9.90E-01	0.40(0.02-7.40)	5.39E-01

Variants are described as minor allele or geno; the contrast allele refers to the minor allele; OR: odds ratio; CI: confidence interval; *P*<sub>unadjusted</sub>: unadjusted P-value from t-test; *P*<sub>adjusted</sub>: P value adjusted using logistic regression analysis with age, HD and hypertension as covariates. F\_Stroke and F\_Control represent the frequency of minor allele or geno in total stroke patients and controls respectively. Significant P values (*P* < 0.05) are in bold and *P*\* < 0.005 (Bonferroni multiple correction threshold).

association analysis was performed using logistic regression with or without adjustment for covariates. A single site association test between rs662 and rs854560 and ischemic stroke was conducted using an allele-based model. Bonferroni correction was undertaken for the 10 SNPs that were adopted into the single site association analysis.

Linkage disequilibrium analysis and haplotype selection were performed using Haploview software with parameter settings for pairwise tagging with *D'* > 0.95 [33]. The Omnibus ANOVA test was conducted using R software [34].

Inverse variance meta-analysis (RevMan 4.0 software) was used to contextualize our studies with two meta-analyses, using the data from PMID: 20856122 [35] and

PMID: 18511872 [30], which also studied the association between rs662 and rs854560 loci and ischemic stroke.

Values of *P* < 0.005 were considered to represent the threshold for statistical significance.

## Results

### Clinical characteristics of total stroke patients and controls

Table 1 shows demographic characteristics and clinical vascular variables in the control and total stroke patients. There were no significant differences in levels of TC, TG and LDL between the controls and total stroke cases. However, HDL levels were significantly lower in stroke

**Table 3 Association between SNPs with ischemic stroke using the additive, dominant, genotype, and the recessive models**

SNP	Model	Allele or geno	F_IS	Control	T-Statistic		Logistic Regression	
					OR (95% CI)	<i>P</i> <sub>unadjusted</sub>	OR (95% CI)	<i>P</i> <sub>adjusted</sub>
rs854571	Additive	C > T	200/660	344/982	0.80(0.65-0.99)	<b>4.34E-02</b>	0.84(0.66-1.07)	1.62E-01
	Dominant	CC + CT/TT	170/330	289/491	0.74(0.56-0.98)	<b>3.79E-02</b>	0.80(0.58-1.10)	1.63E-01
	Recessive	CC/CT + TT	30/330	55/491	0.79(0.50-1.27)	3.31E-01	0.82(0.49-1.37)	4.45E-01
rs13306698	Additive	A > G	62/676	98/988	0.91(0.65-1.29)	6.00E-01	1.07(0.72-1.59)	7.43E-01
	Dominant	AA + AG/GG	61/338	96/494	0.91(0.64-1.30)	6.16E-01	1.09(0.72-1.64)	6.83E-01
	Recessive	AA/AG + GG	1/338	2/494	0.73(0.07-8.08)	7.98E-01	0.55(0.04-7.85)	6.63E-01
rs854572	Additive	C > G	285/668	413/964	0.99(0.82-1.21)	9.44E-01	0.98(0.78-1.23)	8.29E-01
	Dominant	CC + CG/GG	220/334	324/482	0.94(0.70-1.27)	6.87E-01	0.94(0.67-1.32)	7.23E-01
	Recessive	CC/CG + GG	65/334	89/482	1.07(0.75-1.52)	7.21E-01	1.01(0.67-1.53)	9.70E-01
rs7493	Additive	C > G	124/676	176/974	1.02(0.79-1.32)	8.85E-01	0.98(0.73-1.31)	8.82E-01
	Dominant	CC + CG/GG	114/338	163/487	1.01(0.75-1.36)	9.39E-01	0.99(0.71-1.39)	9.68E-01
	Recessive	CC/CG + GG	10/338	13/487	1.11(0.48-2.57)	8.04E-01	0.85(0.34-2.12)	7.23E-01
rs662	Additive	G > A	276/674	356/978	1.19(0.98-1.45)	7.34E-02	1.18(0.94-1.47)	1.46E-01
	Dominant	GG + GA/AA	212/337	282/489	1.25(0.94-1.66)	1.31E-01	1.20(0.86-1.66)	2.84E-01
	Recessive	GG/GA + AA	64/337	74/489	1.32(0.91-1.90)	1.45E-01	1.35(0.88-2.06)	1.67E-01
rs12026	Additive	C > G	124/672	174/978	1.05(0.81-1.36)	7.26E-01	1.02(0.76-1.37)	8.78E-01
	Dominant	CC + CG/GG	114/336	162/389	1.04(0.77-1.39)	8.11E-01	1.04(0.74-1.45)	8.33E-01
	Recessive	CC/CG + GG	10/336	12/489	1.22(0.52-2.86)	6.48E-01	0.95(0.38-2.42)	9.21E-01
rs1053275	Additive	A > G	144/666	186/994	1.19(0.94-1.52)	1.51E-01	1.17(0.89-1.54)	2.70E-01
	Dominant	AA + AG/GG	129/333	165/497	1.27(0.95-1.70)	1.02E-01	1.24(0.89-1.73)	2.10E-01
	Recessive	AA/AG + GG	15/333	21/497	1.07(0.54-2.11)	8.47E-01	1.07(0.49-2.31)	8.68E-01
rs705381	Additive	G > A	74/660	151/990	0.70(0.52-0.95)	<b>2.02E-02</b>	0.65(0.47-0.92)	<b>1.35E-02</b>
	Dominant	GG + GA/AA	65/330	144/395	0.60(0.43-0.83)	<b>2.50E-03*</b>	0.54(0.37-0.79)	<b>1.67E-03*</b>
	Recessive	GG/GA + AA	9/330	7/495	1.96(0.72-5.30)	1.88E-01	1.85(0.60-5.64)	2.83E-01
rs2074353	Additive	A > G	176/662	230/982	1.18(0.94-1.47)	1.53E-01	1.23(0.95-1.60)	1.09E-01
	Dominant	AA + AG/GG	153/331	197/491	1.28(0.97-1.70)	8.30E-02	1.38(1.00-1.91)	5.29E-02
	Recessive	AA/AG + GG	23/331	33/491	1.04(0.60-1.80)	8.99E-01	1.06(0.56-1.99)	8.69E-01
rs854560	Additive	A > T	30/674	39/996	1.14(0.70-1.85)	5.93E-01	1.19(0.69-2.07)	5.36E-01
	Dominant	AA + AT/TT	29/337	38/498	1.14(0.69-1.89)	6.11E-01	1.24(0.70-2.21)	4.57E-01
	Recessive	AA/AT + TT	1/337	1/498	1.48(0.09-23.73)	7.82E-01	0.43(0.02-9.62)	5.92E-01

Variants are described as minor allele or geno and the contrast allele refers to the minor allele; OR: odds ratio; CI: confidence interval; *P*<sub>unadjusted</sub>: unadjusted P-value from t-test; *P*<sub>adjusted</sub>: P value adjusted using logistic regression analysis with age, HD and hypertension as covariates F\_IS and F\_Control represent the frequency of minor allele in ischemic stroke patients and controls respectively. Significant P values (*P* < 0.05) are in bold and *P*\* < 0.005 (Bonferroni multiple correction threshold).

cases than in controls and mean age and incidence of hypertension were significantly higher.

#### Linkage disequilibrium

A total of eleven gene polymorphisms were genotyped in the cases and controls. For *PON1* these included three coding-region polymorphisms (rs662/Q192R, rs13306698/Arg160Gly, and rs854560/Leu55Met) and four regulatory-region polymorphisms (rs705379/-107/-108, rs705381/-160/-162, rs854571/-824/-832, and rs854572/-907/-909). There were also two coding-region polymorphisms of *PON2* (rs12026/Ala148Gly, and rs7493/Ser311Cys), and two coding-region polymorphisms of *PON3* (rs2074353

located in intron and rs1053275/Ala99Ala). The total rate of successful genotyping was 98.6%. All genotype distributions within the studied polymorphisms were in Hardy-Weinberg equilibrium (*P* > 0.05), in both cases and controls, except for rs705379 (-107/-108) (*P* < 0.001), which was located in the promoter of *PON1*.

The results of linkage disequilibrium evaluation analyses are shown in Figure 1A. In this analysis, SNPs with a pairwise *r*<sup>2</sup> > 0.9 were considered to be in the same block. Based on this approach, four haplotype blocks (Block1: rs854560-rs13306698-rs662; Block2: rs854572-rs854571-rs705381; Block3: rs1053275-rs2074353; Block4: rs12026-rs7493) were identified (Figure 1B).

**Table 4 Association between SNPs and hemorrhagic stroke using the additive, dominant, genotype, and the recessive models**

SNP	Model	Allele or geno	F_HS	F_Control	T-Statistic		Logistic Regression	
					OR (95% CI)	<i>P</i> <sub>unadjusted</sub>	OR (95% CI)	<i>P</i> <sub>adjusted</sub>
rs854571	Additive	C > T	92/316	344/982	0.76(0.57-1.00)	5.00E-02	0.76(0.57-1.01)	5.54E-02
	Dominant	CC + CT/TT	78/158	289/491	0.68(0.48-0.98)	3.68E-02	0.70(0.48-1.01)	5.57E-02
	Recessive	CC/CT + TT	14/158	55/491	0.77(0.42-1.43)	4.08E-01	0.71(0.38-1.34)	2.95E-01
rs13306698	Additive	A > G	33/324	98/988	1.03(0.67-1.59)	8.85E-01	1.06(0.68-1.66)	7.93E-01
	Dominant	AA + AG/GG	33/162	96/494	1.06(0.68-1.65)	7.95E-01	1.09(0.69-1.72)	7.06E-01
	Recessive	AA/AG + GG	0/162	2/494	0.00(0.00-inf)	9.99E-01	0.00(0.00-inf)	9.99E-01
rs854572	Additive	C > G	151/320	413/964	1.20(0.93-1.54)	1.73E-01	1.24(0.95-1.61)	1.20E-01
	Dominant	CC + CG/GG	118/160	324/482	1.37(0.92-2.04)	1.23E-01	1.38(0.91-2.08)	1.27E-01
	Recessive	CC/CG + GG	33/160	89/482	1.15(0.73-1.79)	5.46E-01	1.25(0.79-1.99)	3.38E-01
rs7493	Additive	C > G	64/324	176/974	1.12(0.81-1.55)	4.94E-01	1.05(0.75-1.46)	7.77E-01
	Dominant	CC + CG/GG	57/162	163/487	1.08(0.74-1.57)	6.90E-01	1.03(0.70-1.51)	8.94E-01
	Recessive	CC/CG + GG	7/162	13/487	1.65(0.65-4.20)	2.97E-01	1.28(0.49-3.36)	6.13E-01
rs662	Additive	G > A	108/324	356/978	0.88(0.68-1.14)	3.36E-01	0.85(0.65-1.12)	2.48E-01
	Dominant	GG + GA/AA	88/162	282/489	0.87(0.61-1.25)	4.56E-01	0.82(0.56-1.18)	2.85E-01
	Recessive	GG/GA + AA	20/162	74/489	0.79(0.47-1.34)	3.83E-01	0.80(0.46-1.38)	4.26E-01
rs12026	Additive	C > G	64/322	174/978	1.15(0.83-1.59)	3.95E-01	1.10(0.78-1.53)	5.92E-01
	Dominant	CC + CG/GG	57/161	162/389	1.11(0.76-1.61)	5.96E-01	1.07(0.72-1.57)	7.44E-01
	Recessive	CC/CG + GG	7/161	12/489	1.81(0.70-4.67)	2.22E-01	1.47(0.55-3.92)	4.39E-01
rs1053275	Additive	A > G	58/318	186/994	0.97(0.71-1.33)	8.55E-01	0.96(0.70-1.33)	8.07E-01
	Dominant	AA + AG/GG	49/159	165/497	0.90(0.61-1.32)	5.77E-01	0.91(0.61-1.35)	6.36E-01
	Recessive	AA/AG + GG	9/159	21/497	1.36(0.61-3.03)	4.53E-01	1.17(0.51-2.69)	7.09E-01
rs705381	Additive	G > A	32/312	151/990	0.62(0.41-0.94)	2.42E-02	0.62(0.40-0.95)	2.90E-02
	Dominant	GG + GA/AA	30/156	144/395	0.58(0.37-0.90)	1.61E-02	0.57(0.36-0.90)	1.65E-02
	Recessive	GG/GA + AA	2/156	7/495	0.91(0.19-4.40)	9.02E-01	1.22(0.24-6.22)	8.13E-01
rs2074353	Additive	A > G	74/340	230/982	0.99(0.74-1.32)	9.57E-01	0.96(0.72-1.29)	8.05E-01
	Dominant	AA + AG/GG	63/159	197/491	0.98(0.68-1.41)	9.11E-01	0.98(0.67-1.42)	8.98E-01
	Recessive	AA/AG + GG	11/159	33/491	1.03(0.51-2.09)	9.31E-01	0.87(0.42-1.82)	7.21E-01
rs854560	Additive	A > T	9/324	39/996	0.70(0.34-1.46)	3.46E-01	0.57(0.26-1.25)	1.58E-01
	Dominant	AA + AT/TT	9/162	38/498	0.71(0.34-1.51)	3.74E-01	0.57(0.26-1.28)	1.74E-01
	Recessive	AA/AT + TT	0/162	NA	NA	NA	NA	NA

Variants are described as minor allele or geno and the contrast allele refers to the minor allele; *P*<sub>unadjusted</sub>: unadjusted P-value from t-test; *P*<sub>adjusted</sub>: P value adjusted using logistic regression analysis with age, HD and hypertension as covariates. F\_HS and F\_Control represent the frequency of minor allele in hemorrhagic stroke patients and controls respectively. NA means not applicable. Significant P values (*P* < 0.05) are in bold.

### Single site association

The association between the ten SNPs included in the four blocks and total stroke occurrence was analyzed using additive, dominant, genotype, and recessive models. As shown in Table 2, two polymorphisms, rs705381 and rs854571 were significantly associated with total stroke using additive and dominant models. The allele A of rs705381 and the allele T of rs854571 were both less frequent in patients with total stroke than in controls. The association remained significant after logistic regression analysis adjusting for age, HDL and hypertension using the additive model (rs705381, *P*<sub>adjusted</sub> = 0.0058, OR = 0.67 [95% CI = 0.50-0.89]; and rs854571, *P*<sub>adjusted</sub> = 0.0330, OR

= 0.80 [95% CI = 0.65-0.98]). However, both P-values failed to reach significance after the Bonferroni adjustment for multiple comparisons. Analysis using the dominant model, showed that the differences in rs705381 remained significant after Bonferroni correction (*P*<sub>adjusted</sub> = 0.0007, OR = 0.57 [95% CI = 0.41-0.79]), but the differences in rs854571 did not. There was no significant association between any of the SNPs of PON genes and total strokes when analyzed using the recessive model.

As shown in Table 3, rs705381 was significantly associated with ischemic stroke after adjustment of confounders in both additive and dominant models (*P*<sub>adjusted</sub> = 0.0017, OR = 0.54 [95% CI = 0.37-0.79]).

**Table 5 Haplotypes of the four blocks between total strokes and control subjects**

Haplotype	Logistic Regression			
	OR	<i>P</i> <sub>unadjusted</sub>	OR	<i>P</i> <sub>adjusted</sub>
<b>Block1: rs854560-rs13306698-rs662</b>				
OMNIBUS	NA	0.9371	NA	0.9569
TAA	1.03	0.8820	0.95	0.8390
AAA	1.08	0.4170	1.06	0.5790
AGG	0.95	0.7640	0.98	0.8840
AAG	0.94	0.4810	0.96	0.6580
<b>Block2: rs854572-rs854571-rs705381</b>				
OMNIBUS	NA	<b>0.0129</b>	NA	<b>0.0394</b>
CTT	1.05	0.6170	1.08	0.4200
CTC	0.64	<b>0.0013</b>	0.65	<b>0.0037</b>
GCC	0.99	0.9110	0.99	0.9280
CCC	1.24	<b>0.0442</b>	1.19	0.1420
<b>Block3: rs1053275-rs2074353</b>				
OMNIBUS	NA	0.4970	NA	0.5757
GG	1.10	0.3970	1.10	0.4210
AG	1.09	0.6630	1.13	0.5880
AA	0.90	0.2920	0.89	0.3010
<b>Block4: rs12026-rs7493</b>				
OMNIBUS	NA	0.2479	NA	0.5467
GG	1.08	0.5390	1.03	0.8430
CC	0.92	0.5020	0.96	0.7660

Haplotypes observed in <1% of the control subjects are not listed in the table. OR: odds ratio; *P*<sub>unadjusted</sub>: unadjusted P-value from t-test; *P*<sub>adjusted</sub>: P value adjusted using logistic regression analysis with age, HD and hypertension as covariates. OMIBUS value was calculated by an ANOVA analysis for including or not including the haplotype information in a likelihood ration test of nested model. The OR in one block for each haplotype was calculated by using all the other haplotypes in the same block as the reference haplotype. Significant P values (*P* < 0.05) are in bold.

However, no significant association with ischemic stroke was found using the recessive model.

Rs854571 was associated with hemorrhagic stroke, with marginal significance (*P*<sub>unadjusted</sub> = 0.0500, OR = 0.76 [95% CI = 0.57-1.00]) using the additive model, and rs705381 showed a significant association in both additive (*P*<sub>adjusted</sub> = 0.0290, OR = 0.62 [95% CI = 0.40-0.95]) and dominant models (*P*<sub>adjusted</sub> = 0.0165, OR = 0.57 [95% CI = 0.36-0.90]) (Table 4). However, neither of the two SNPs was significantly associated with hemorrhagic stroke after the Bonferroni correction. Thus, there was no significant finding for hemorrhagic stroke with any of the three models.

#### Haplotype analysis

Haplotype analysis conducted in the four blocks, with or without adjustment for age, HDL and hypertension as covariates is shown in Table 5. Block 2 consisting of rs854572, rs854571 and rs705381 was associated with total stroke (*P* = 0.0129 Omnibus test), and included one protective

haplotype C-T-C (OR = 0.64; *P*<sub>unadjusted</sub> = 0.0013,) and one nominal risk haplotype C-C-C (OR = 1.24; *P*<sub>unadjusted</sub> = 0.0442,). The association for haplotype C-T-C remained significant after adjustment for age, HDL and hypertension as covariates (OR = 0.65; *P* = 0.0037). No other significant haplotype associations were found.

#### Meta-analysis

Two meta-analyses, PMID: 20856122 [35] and PMID: 18511872 [30], which studied the association between rs662 and rs854560 loci and ischemic stroke were contextualized with our study using the random effects model. Forests plot for rs662 from 25 studies including our own are shown in Figure 2. There was a nominal significant association between rs662 and ischemic stroke (*P* = 0.0100, OR = 1.08 [95% CI = 1.02-1.15]) yielding 1.08 per G allele copy, with no statistical evidence for statistical heterogeneity (*P* = 0.0400, *I*<sup>2</sup> = 36%) between studies.

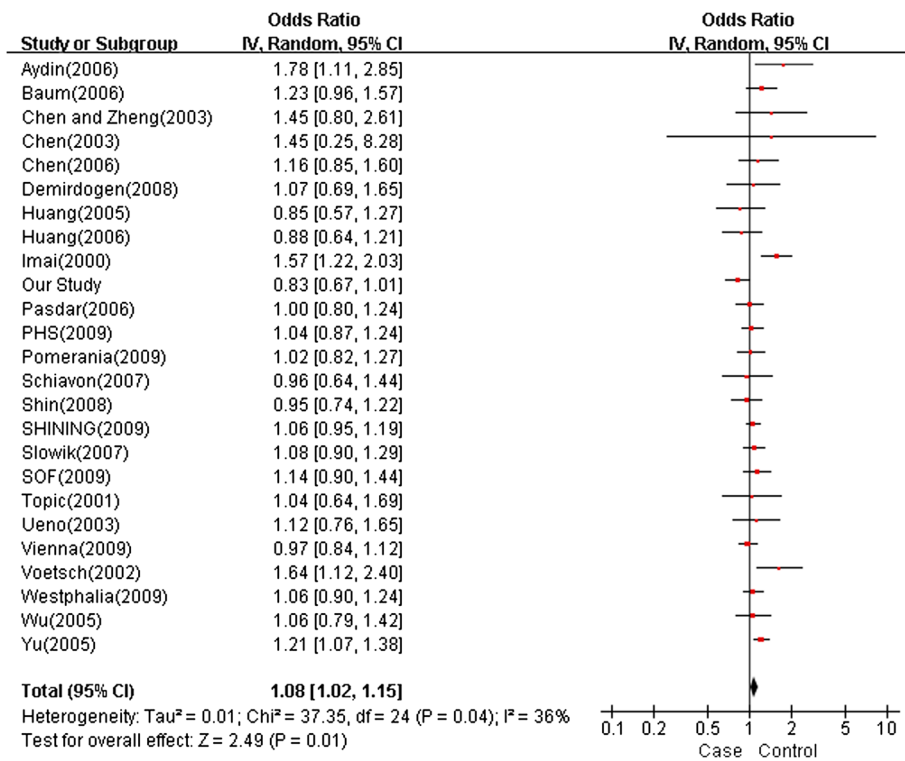
There was no evidence of an association between rs854560 and ischemic stroke risk (*P* = 0.3700, OR = 0.97 [95% CI = 0.91-1.04]) and no evidence of heterogeneity (*P* = 0.2700, *I*<sup>2</sup> = 16%) between studies (Figure 3).

#### Discussion

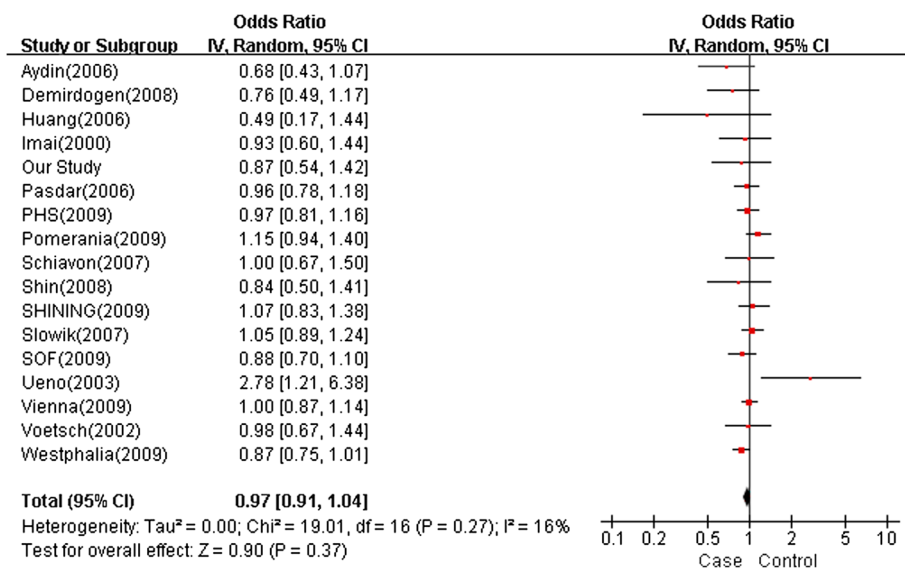
The present study investigated the association of 11 polymorphisms in 3 *PON* genes with the risk of stroke. Using a dominant model, we demonstrated that rs705381 (-162) was significantly associated with total stroke and ischemic stroke but not with hemorrhagic stroke. There was also a nominal association between rs854571 (-824) and stroke with the allele T as a protective factor.

Both rs705381 and rs854571 polymorphisms located in the promoter region of *PON1* were associated with stroke, which was consistent with previous findings [19,36-39]. The protective effect of -162 T polymorphism on total stroke and ischemic stroke was also consistent with previous observations [40] which suggested that NF-1, a ubiquitous nuclear factor and a transcriptional activator, has a binding site on *PON1* if allele A appears at -162. Other studies have shown that -162 T polymorphism results in higher expression levels of *PON1* [40,41] There is also evidence to suggest a correlation between AA (-162) and high PON activities in Caucasians [42].

Our results support the hypothesis that the protective effect of -162 T polymorphism might be attributable to high PON activity [42]. We also found weak evidence to suggest that -824 T was associated with a reduced propensity to suffer stroke. However, the evidence was no longer apparent after Bonferroni correction for multiple comparisons. It has been previously reported that -824 T (824A in their finding) was associated with low serum PON levels [43]. Negative associations between -162 and -824 have been reported in studies in American populations [23,40]. These findings highlight the potential influence of



**Figure 2** Meta-analysis of studies investigating the association of PON1 rs662 with ischemic stroke using a random effects model. The point estimate of the OR (square proportional to the weight of each study) and 95% CI for the OR (extending lines) for each study. The summary OR and 95% CIs by random effects calculations are depicted as a diamond. Values higher than 1 indicate that the G allele is associated with increased risk of ischemic stroke.



**Figure 3** Meta-analysis of studies investigating the association of PON1 rs854560 with ischemic stroke using a random effects model. Values higher than 1 indicate that the A allele is associated with increased risk of ischemic stroke risk. The layout is the same as that in Figure 2.



ethnic differences in terms of the founder effect and identical-by-descent principles [44,45].

Patients with coronary heart disease (CHD) have been shown to have a higher frequency of -162 T allele than the controls, suggesting allele A may be associated with risk of CHD in the Han Chinese population [46]. However, in our study, we found a protective effect of the -162 T polymorphism on stroke.

Haplotype analysis further confirmed our positive results and identified a positive association between the protective haplotype C-T-C and the risk haplotype C-C-C of rs854572-rs854571-rs705381 (Block 2) with total stroke. No significant associations were observed for stroke susceptibility with the two coding region polymorphisms in *PON2*, which was consistent with previous findings in the Han Chinese population and in North Americans [24,29], although a positive association of Ser311Cys was found in a Polish population [22].

The absence of any positive correlations between stroke risk and the two *PON3* polymorphisms in our study was also consistent with reported findings in Caucasian and North American patients [24,27].

Our study was conducted in a relatively large Chinese sample pool and included careful assessment of two stroke subtypes. We also selected common variants in all three members of the PON gene family. However, functional detection of PON activities was not undertaken in the present study and investigation of the association between SNPs and large or small vessel strokes was not possible as a complete classification of the subtype of ischemic stroke subjects was not available in our study. In our study, results from both adjusted and unadjusted analyses were in line with each other. However, in other settings, authorities have discouraged the use of data adjustments for the determination of the total genetic effect [47]. It, therefore, remains uncertain as to whether adjusted or unadjusted data should be used to interpret our results in clinical context.

## Conclusion

The study identified rs705381 (-162) as being significantly associated with total stroke and ischemic stroke, and demonstrated a weak association for rs854571 (-824) in the Han Chinese population. These findings support the involvement of *PON* polymorphisms in the development of stroke. Further studies with larger sample sizes are required to validate these findings and to elucidate the underlying biological mechanisms.

## Competing interests

The authors have no competing interests.

## Authors' contributions

YS and YS designed the study, coordinated sample recruitment and revised the final manuscript. GZ participated in study design and collected the samples. WL drafted the manuscript and carried out the statistical analysis.

ZL helped with the statistical analysis and draft manuscript preparation. HL, RM and XK helped with the sample collection. YR and XL performed the SNP genotyping. All authors read and approved the final manuscript.

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