Susceptibility of *PON1/PON2* Genetic Variations to Ischemic Stroke Risk in a Chinese Han Population

This article was published in the following Dove Press journal:
Pharmacogenomics and Personalized Medicine

Yuqin Pan^{1,*}
Bangshun He^{1,*}
Huiling Sun¹
Tao Xu¹
Bei Pan¹
Shukui Wang¹
Yanping Mei²

¹General Clinical Research Center, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu Province, 210006, People's Republic of China;

²Department of Laboratory Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, People's Republic of China

*These authors contributed equally to this work

Background: Paraoxonases (PONs) are a family of orphan enzymes with multiple functions, including anti-inflammatory, antioxidative, antiatherogenic activities. Studies have suggested that genetic variations in *PON1* and *PON2* are associated with ischemic stroke (IS) risk; however, the conclusion remains unclear in the Chinese population.

Methods: To investigate the susceptibility of genetic variations in *PON1* and *PON2* to risk of IS and its subtypes, this case-control study was carried out on a Chinese population comprising 300 IS patients and 300 healthy controls. Genotypes of six genetic variations in *PON1* and *PON2* were identified with an improved multiplex ligase detection-reaction technique.

Results: *PON1* rs662 was associated with increased risk of IS (CT vs. TT — OR_{adjusted} 1.79, 95% CI 1.08–2.97; $p=0.025$). Stratified analysis for patients by sex revealed that the significant association of *PON1* rs662 with IS risk was maintained in the male cohort (CT vs. TT — OR_{adjusted} 2.59, 95% CI 1.29–5.21 [$p=0.009$]; CT/CC vs. TT — OR_{adjusted} 2.03, 95% CI 1.05–3.93 [$p=0.036$]), but not in the female cohort. Analysis according to IS subtype revealed that *PON1* rs662 genetic variation was an increased risk in the subcohort of patients with large-artery atherosclerosis (CT/CC vs. TT — OR_{adjusted} 2.31, 95% CI 1.09–4.91; $p=0.029$), but not in patients with other types of IS.

Conclusion: This study suggested that *PON1* rs662 presented a potential risk of IS, especially for males, and this association was more obvious for large-artery atherosclerosis.

Keywords: ischemic stroke, genetic variation, *PON1*, *PON2*

Introduction

Stroke has become one of the main causes of death and disability worldwide: >15 million people suffer ischemic stroke (IS), each year, causing 6 million deaths and 5 million disabilities. It is the second-leading cause of disability and death in the people >60 years old and the fifth-leading cause of death in people aged 15–59 years.¹ IS incidence varies depending on age, sex, race, and genetic factors. Epidemiological studies have revealed several factors, including age, sex, obesity, cigarette smoking, hypertension, diabetes mellitus, atherosclerosis, and dyslipidemias, contribute to the occurrence of stroke.^{2–5}

IS, hemorrhagic stroke, and transient ischemic attacks are three main types of cerebrovascular events. Of these, IS occurs as a result of an obstruction within a blood vessel supplying blood to the brain and is the most common form of cerebrovascular disease, accounting for approximately 87% of all stroke cases. Actually, the etiology of IS affects risk of recurrence, disease prognosis, and choices for management. Therefore, the categorization of subtypes of IS based mainly on etiology was developed for the TOAST study,⁶ which developed five subtypes of IS: 1) large-artery

Correspondence: Shukui Wang
General Clinical Research Center, Nanjing First Hospital, Nanjing Medical University, 68 Changle Road, Nanjing, Jiangsu 210006, People's Republic of China
Email shukuiwang@163.com

Yanping Mei
Department of Laboratory Medicine, Nanjing First Hospital, Nanjing Medical University, 68 Changle Road, Nanjing, Jiangsu 210006, People's Republic of China
Email 4940556@qq.com

atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, 4) stroke of other determined etiology, and 5) stroke of undetermined etiology.

Studies on stroke etiology have indicated that a complex interaction of genetic and environmental factors contributes to the occurrence of stroke.^{7,8} For genetic factors, a number of genes involved in cholesterol metabolism, inflammation, blood coagulation, homocysteine metabolism, and the renin-angiotensin system have been suggested to contribute to the development of stroke.⁹ Specifically, genes involved in lipid metabolism have been implicated in the etiology of stroke insofar as the concentration of low-density lipoprotein (LDL) and the oxidation of LDL represent initial events in atherogenesis by producing proatherosclerotic and proinflammatory oxidized lipids, whereas high-density lipoprotein (HDL) in functional form is an atheroprotective factor through its multifunctionality, including reverse cholesterol transport and antioxidant, anti-inflammatory, and antithrombotic effects.

Paraoxonases (PONs), a family of orphan enzymes with multiple activities, are tightly associated with the HDL surface that decreases the peroxidation of LDL and have anti-inflammatory, antioxidative, and antiatherogenic activities. *PON1*, *PON2*, and *PON3* are three known members of this gene family, on the long arm of chromosome 7 between q21.3 and q22.1 in humans. These three types of PONs are antiatherogenic enzymes in terms of their antioxidant activities and inhibiting the oxidation of LDL, along with preventing oxidative modification of the cell membrane.¹⁰ Of these, PON1 is a calcium-dependent glycoprotein associated with HDL particles, and exerts a cardioprotective function through its hydrolyzing effect on LDL-oxidized phospholipids. Studies have revealed that genetic variations in *PON1* can affect its concentration or activity and predict the risk of IS.¹¹ Genetic variations in the promoter region of *PON1* and the coding regions of *PON1* and *PON2* have been focused on for their susceptibility to IS; however, the results were not consistent,¹² which may be attributed to differences in genetic background among ethnicities. To investigate the susceptibility of genetic variations in *PON1* and *PON2* to the risk of IS and its subtype, this case-control study was carried out on a Chinese cohort.

Methods

Study Subjects

A total of 300 patients were enrolled as cases, and all patients were diagnosed with IS on the basis of clinical symptoms, physical examination, and brain computed

tomography or magnetic resonance imaging, independently assessed by a technologist and a physician. All the patients suffered 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 attacks, hemorrhagic stroke, cerebral trauma, cardiogenic thrombosis, cerebrovascular malformations, coagulation disorders, autoimmune diseases, tumors, peripheral vascular disease, or chronic infection diseases were excluded. According to the criteria and characteristics of the enrolled patients, we divided the patients into four subtypes: 1) large-artery atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, and 4) stroke of other etiology.

Healthy control subjects were recruited from the Health Medical Center of Nanjing First Hospital during the same period. All these were confirmed as healthy according to the results of routine health examination and matched to cases in terms of age and sex. For the healthy controls, those with history of tumors, autoimmune diseases, genetic diseases, liver ailments, and hematologic diseases were excluded. Demographic characteristics and clinical information — including sex, age, drinking, smoking, diastolic blood pressure (DBP), systolic blood pressure (SBP), diabetes, fasting serum levels of total plasma cholesterol, triglycerides (TGs), HDL, LDL, glucose, and homocysteine — were abstracted from medical records at our hospital. All enrolled participants were heritably unrelated ethnic Han Chinese from the same geographic region: Nanjing City, Jiangsu, China. The protocol of this study was in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Nanjing First Hospital, and written informed consent was obtained from all participants.

SNP Selection and Genotyping

All potential genetic variations in *PON1* or *PON2* associated with risk of IS were retrieved from the National Center for Biotechnology Information dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>), and then potential genetic variations were selected: 1) positioned in exons, promoter regions, 5'UTRs, 3'UTRs, or splice sites; 2) minor-allele frequency $\geq 5\%$; and 3) had been reported to be associated with IS risk, previously. Finally, six genetic variations in *PON1* and *PON2* were selected (see Table 1 for details). Blood samples were collected from all participants with EDTA-coated tubes and stored in a refrigerator at -80°C . Total DNA was extracted from whole-blood samples and concentrated

Table 1 Enrolled Genetic Variations

SNP ID	Region	Allele	Chromosome	Position	Gene
rs705381	Promoter	-162A/G	7	94953949	PON1
rs854571	Promoter	-832C/T	7	94954619	PON1
rs854572	Promoter	-909G/C	7	94954696	PON1
rs3735590	3'UTR	+26080 T/C	7	94927495	PON1
rs662	Exon 6, Q192R	+16342 C/T	7	94937446	PON1
rs7493	Exon 9, C311S	+34610C/G	7	95034775	PON2

using a mini whole blood genomic DNA purification kit (GoldMag Xi'an, China) according to the manufacturer's instructions, and then DNA purity was measured with spectrometry (DU530; Beckman Instruments, Fullerton, CA, US).

All six genetic variations selected were genotyped using the improved multiplex ligase detection-reaction technique developed by Genesky Biotech (Shanghai, China). In brief, multiplex polymerase chain reaction was performed to amplify genetic-variation loci. Secondly, amplification products were purified by nuclease and shrimp alkaline enzyme. Finally, a connection the reaction was performed to have each site containing two 5' terminal allele-specific probes and a 3' terminal-specific probe of fluorescent tags, and then ligation products were analyzed with an ABI 3730XL. Of all subjects, 10% were randomly selected and subjected to repeated genotyping, and reproducibility of 100% attained.

Statistical Analysis

Differences in demographic characteristics between patients and controls were compared by univariate analysis with the use of Student's *t*-test. Hardy-Weinberg equilibrium in the healthy control group was tested using a goodness-of-fit χ^2 test. Logistic regression was applied to calculate ORs and 95% CIs. The dominant, codominant, and additive models were tested for all genetic variations. $p < 0.05$ was considered statistically significant.

Results

A total of 300 patients with IS and 300 age- and sex-matched healthy controls were enrolled in this population-based case-control association study, and their demographic data and clinical characteristics are summarized in Table 2. There were no significant differences with respect to age ($p=0.136$), sex ($p=0.273$), smoking ($p=0.351$), or drinking ($p=0.854$) between the two groups. For clinical characteristics, levels of DBP ($p<0.001$), SBP ($p<0.001$), TGs ($p=0.024$), Glu ($p<0.001$), and homocysteine ($p=0.021$)

were significantly higher in patients than in controls. In contrast, levels of HDL in patients were significantly lower than in controls ($p<0.001$), as shown in Table 2. A total of 117 patients were identified as having large-artery

Table 2 Demographic Data and Clinical Characteristics of Patients with Ischemic Stroke and Controls

	Patients, n (%)	Controls, n (%)	p-value
Total cases	300	300	
Age (mean $\pm$ SD, years)	68.23 $\pm$ 11.33	66.95 $\pm$ 9.60	0.136
Sex			0.273
Male	181 (60.33)	194 (64.67)	
Female	119 (39.67)	106 (35.33)	
Smoking			0.351
Yes	47 (15.67)	39 (87.00)	
No	253 (84.33)	261 (13.00)	
Drinking			0.854
Yes	15 (5.00)	16 (5.33)	
No	285 (95.00)	284 (94.67)	
SBP (mean $\pm$ SD)	142.73 $\pm$ 18.86	124.52 $\pm$ 13.08	<0.001
DBP (mean $\pm$ SD)	83.60 $\pm$ 10.68	75.72 $\pm$ 8.15	<0.001
TC (mean $\pm$ SD)	4.73 $\pm$ 1.52	4.65 $\pm$ 0.94	0.438
TGs (mean $\pm$ SD)	1.47 $\pm$ 0.86	1.34 $\pm$ 0.57	0.024
LDL (mean $\pm$ SD)	2.73 $\pm$ 0.85	2.68 $\pm$ 0.62	0.405
HDL (mean $\pm$ SD)	1.13 $\pm$ 0.37	1.39 $\pm$ 0.29	<0.001
Glu (mean $\pm$ SD)	6.41 $\pm$ 2.65	5.41 $\pm$ 0.72	<0.001
Hyc (mean $\pm$ SD)	16.77 $\pm$ 7.23	15.70 $\pm$ 3.38	0.021
Clinical stage			
Small-vessel occlusion	56 (18.67)		
Large-artery atherosclerosis	117 (39.00)		
Cardioembolism	28 (9.33)		
Other	97 (32.33)		

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total plasma cholesterol; TGs, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Glu, glucose; Hyc, homocysteine.

Table 3 Genotype Distribution of Polymorphisms in all Participants

	Patients, n (%)	Controls, n (%)	OR (95% CI)	p-value
rs705381				
CC	244 (81.33)	231 (77.00)	Reference	
CT	53 (17.67)	63 (21.00)	0.76 (0.50–1.15)	0.194
TT	3 (1.00)	6 (2.00)	0.43 (0.11–1.76)	0.241
CT/TT	56 (18.67)	65 (23.00)	0.74 (0.49–1.10)	0.133
rs854571				
CC	153 (51.00)	152 (50.67)	Reference	
CT	124 (41.33)	121 (40.33)	1.03 (0.73–1.45)	0.861
TT	23 (7.67)	27 (9.00)	0.82 (0.45–1.50)	0.517
CT/TT	147 (49.00)	148 (49.33)	0.99 (0.72–1.37)	0.957
rs854572				
CC	89 (29.67)	93 (31.00)	Reference	
CG	152 (50.67)	145 (48.33)	1.11 (0.77–1.61)	0.581
GG	59 (19.67)	62 (20.67)	0.98 (0.61–1.57)	0.939
CG/GG	211 (70.33)	207 (69.00)	1.07 (0.75–1.51)	0.720
rs3735590				
GG	223 (74.33)	231 (77.00)	Reference	
GA	70 (23.33)	64 (21.33)	1.17 (0.79–1.73)	0.428
AA	7 (2.33)	5 (1.67)	1.28 (0.40–4.15)	0.676
GA/AA	77 (25.67)	69 (23.00)	1.19 (0.82–1.74)	0.365
rs0662				
TT	34 (11.33)	48 (16.00)	Reference	
CT	154 (51.33)	129 (43.00)	1.79 (1.08–2.97)	0.025
CC	112 (37.33)	123 (41.00)	1.47 (0.87–2.50)	0.153
CT/CC	266 (88.67)	252 (84.00)	1.58 (0.98–2.55)	0.063
rs7493				
GG	200 (66.67)	193 (64.33)	Reference	
CG	87 (29.00)	92 (30.67)	0.93 (0.65–1.33)	0.678
CC	13 (4.33)	15 (5.00)	0.85 (0.39–1.85)	0.684
CG/CC	100 (23.33)	107 (35.67)	0.91 (0.65–1.28)	0.602

atherosclerosis, 28 with cardioembolism, 56 with small-ves-
sel occlusion, and 97 with other etiologies (Table 2).

Observed frequencies of all tested genotypes in con-
trols were not derived from the Hardy–Weinberg equi-
librium (data not shown). Logistic regression analysis
revealed that *PON1* rs662 genetic variation was associated
with increased risk of IS (CT vs. TT — OR_{adjusted} 1.79,
95% CI 1.08–2.97; $p=0.025$), as shown in Table 3.
Subgroup analysis of patients stratified by sex revealed a
significant association of *PON1* rs662 with IS risk was in
the male cohort (CT vs. TT — OR_{adjusted} 2.59, 95% CI
1.29–5.21 [$p=0.009$; CT/CC vs TT — OR_{adjusted} 2.03, 95%
CI 1.05–3.93, [$p=0.036$]) but not in the of female cohort,
as shown in Table 4. Further, we evaluated the suscepti-
bility of genetic variations to risk of subtypes of IS. *PON1*

rs662 showed increased risk inpatients with large-artery
atherosclerosis (CT/CC vs. TT — OR_{adjusted} 2.31, 95% CI
1.09–4.91; $p=0.029$) but not in patients with any other type
of IS, suggesting the risk of *PON1* rs662 for IS is modified
by its type, as shown in Table 5.

Discussion

This population-based case–control association study with
300 paired cases and controls revealed that the *PON1*
rs662 genetic variation was potentially associated with
increased risk of IS, especially in the male population,
and that the susceptibility of *PON1* rs662 to IS risk
could be modified by its etiology. We observed that geno-
types in controls were not derived from the Hardy–
Weinberg equilibrium, indicating controls in this study

Table 4 Genotype Distribution of Polymorphisms in all Participants Stratified by Sex

	Male			Female		
	Patients/controls (%)	OR (95% CI) ^a	p-value ^a	Patients/controls (%)	OR (95% CI) ^a	p-value ^a
rs705381						
CC	151/159 (83.43/81.96)	Reference		93/72 (78.15/67.92)	Reference	
CT	28/33 (15.47/17.1)	0.88 (0.51–1.54)	0.656	25/30 (20.01/28.3)	0.56 (0.30–1.06)	0.073
TT	2/2 (1.10/1.03)	1.07 (0.15–7.82)	0.950	1/4 (0.84/3.77)	0.15 (0.02–1.36)	0.091
CT/TT	30/35 (16.57/18.04)	0.89 (0.52–1.53)	0.681	26/34 (21.85/32.08)	0.50 (0.27–0.94)	0.030
rs854571						
CC	94/107 (51.93/55.15)	Reference		59/45 (49.58/42.45)	Reference	
CT	73/72 (40.33/37.11)	1.20 (0.78–1.85)	0.413	51/49 (42.86/46.23)	0.83 (0.47–1.48)	0.526
TT	14/15 (7.73/7.73)	1.08 (0.49–2.36)	0.852	9/12 (7.56/11.32)	0.59 (0.22–1.58)	0.291
CT/TT	87/87 (48.07/44.85)	1.16 (0.77–1.76)	0.469	60/61 (50.42/57.55)	0.79 (0.46–1.36)	0.391
rs854572						
CC	53/58 (29.28/29.90)	Reference		36/35 (30.25/33.02)	Reference	
CG	89/89 (49.17/45.88)	1.10 (0.68–1.78)	0.687	63/56 (52.94/52.83)	1.12 (0.61–2.07)	0.710
GG	39/47 (21.55/24.23)	0.81 (0.45–1.45)	0.475	20/15 (16.81/14.15)	1.27 (0.55–2.94)	0.569
CG/GG	128/136 (70.72/70.71)	1.00 (0.64–1.56)	0.987	83/71 (69.75/66.98)	1.17 (0.65–2.09)	0.607
rs3735590						
GG	127/148 (70.17/76.29)	Reference		96/83 (80.67/78.3)	Reference	
GA	52/43 (28.73/22.16)	1.39 (0.87–2.23)	0.175	18/21 (15.13/19.81)	0.76 (0.37–1.55)	0.442
AA	2/3 (1.10/1.55)	0.76 (0.12–4.75)	0.769	5/2 (4.20/1.89)	4.32 (0.48–38.95)	0.193
GA/AA	54/46 (29.83/23.71)	1.35 (0.85–2.14)	0.203	23/23 (19.33/21.70)	0.92 (0.47–1.81)	0.807
rs662						
TT	15/31 (8.29/15.98)	Reference		19/17 (15.97/16.04)	Reference	
CT	95/76 (52.49/39.18)	2.59 (1.29–5.21)	0.008	59/53 (49.58/50.00)	1.06 (0.48–2.35)	0.891
CC	71/87 (39.23/44.85)	1.66 (0.82–3.38)	0.159	41/36 (34.45/33.96)	1.29 (0.55–3.04)	0.565
CT/CC	166/163 (91.71/84.02)	2.03 (1.05–3.93)	0.036	100/89 (84.03/83.96)	1.17 (0.55–2.49)	0.684
rs7493						
GG	120/126 (66.30/64.95)	Reference		80/67 (67.23/63.21)	Reference	
CG	53/57 (29.28/29.38)	1.05 (0.66–1.65)	0.851	34/35 (28.57/33.02)	0.73 (0.40–1.32)	0.294
CC	8/11 (4.42/5.67)	0.80 (0.31–2.08)	0.648	5/4 (4.20/3.77)	1.07 (0.24–4.74)	0.929
CG/CC	61/68 (33.70/35.05)	1.00 (0.65–1.55)	0.991	39/39 (32.77/36.79)	0.76 (0.43–1.34)	0.378

Note: ^aAdjusted for age, sex, smoking, and drinking.

were a large, randomly matching population with no selection, genetic drift, migration, or mutation, suggesting the controls we selected were reliable.

PON1 rs662 is a genetic variation in the coding region of *PON1*, causing a missense substitution at position 192 (¹⁹²Gln [Q]/Arg [R]). Studies have reported that this genetic variation is the major determining factor leading to *PON1* activity in that the 192R variant can hydrolyze paraoxonase faster than the 192Q variant.^{13–14} Therefore, *PON1* rs662 has been regarded as a risk factor of cardiovascular disease¹⁵ and IS, although results have often been conflicting.¹¹ This study based on a Chinese population also reported the *PON1* rs662 R(G) allele was a potential risk factor for IS, consistent with pooled results of published data verifying

the association between *PON1* rs662 and stroke risk,^{16,17} especially in Asian populations.¹² In addition, we observed that the risk of *PON1* rs662 in IS was more obvious in the male subcohort than the female one, indicating the interaction of sex and *PON1* rs662 contributed to different risk of IS^{18,19} and that males with the *PON1* rs662 C allele are at higher risk of IS. Consistently, sex differences, including dyslipidemia, are regarded as predictors of IS,²⁰ which may contribute to the sex difference in susceptibility of *PON1* rs662 to IS. Despite the limited sample size, we observed the risk of *PON1* rs662 for IS was more obvious in patients with large-artery atherosclerosis, consistent with the result of previous report.²¹ Actually, large-artery atherosclerosis shares a similar etiology with atherosclerosis,⁶ and *PON1*

Table 5 Associations Between Genetic Variations and Risk of Types Of Ischemic Stroke

	Small-vessel Occlusion		Large-Artery Atherosclerosis		Cardioembolism		Other Etiology	
	OR (95% CI) ^a	p-value	OR (95% CI) ^a	p-value	OR (95% CI) ^a	p-value	OR (95% CI) ^a	p-value
rs705381 CC CT/TT	Reference 0.70 (0.33–1.48)	0.353	Reference 0.64 (0.36–1.13)	0.125	Reference 0.61 (0.23–1.66)	0.333	Reference 0.88 (0.50–1.54)	0.647
rs854571 CC CT/TT	Reference 1.04 (0.58–1.86)	0.892	Reference 1.05 (0.68–1.62)	0.826	Reference 0.91 (0.41–2.04)	0.824	Reference 0.88 (0.56–1.40)	0.600
rs854572 CC CG/GG	Reference 1.35 (0.70–2.61)	0.365	Reference 1.17 (0.72–1.89)	0.527	Reference 0.58 (0.26–1.30)	0.184	Reference 1.04 (0.63–1.72)	0.867
rs3735590 GG GA/AA	Reference 1.36 (0.72–2.59)	0.346	Reference 1.24 (0.75–2.04)	0.398	Reference 1.00 (0.38–2.64)	1.000	Reference 0.98 (0.57–1.70)	0.938
rs662 TT CT/CC	Reference 1.16 (0.51–2.62)	0.728	Reference 2.31 (1.09–4.91)	0.029	Reference 0.71 (0.27–1.84)	0.480	Reference 1.76 (0.85–3.66)	0.131
rs7493 GG CG/CC	Reference 0.68 (0.36–1.30)	0.243	Reference 1.29 (0.82–2.01)	0.270	Reference 0.92 (0.40–2.11)	0.835	Reference 0.72 (0.43–1.18)	0.193

Note: ^aAdjusted for age, sex, smoking, and drinking.

rs662 genetic variation presents a risk of atherosclerosis.²² Therefore, the contradiction of published data regarding the susceptibility of *PON1* rs662 to IS risk may be due to lack of classification of stroke subtypes, which should be verified by further larger studies. To date, few studies have actually discussed the association between *PON1* rs662 polymorphism and risk of IS subtypes. The novelty of this study was that we firstly reported that *PON1* rs662 polymorphism was associated with risk of large-artery atherosclerosis in a Chinese population, although the sample was relatively small.

Three genetic variations in the promoter and one in 3' UTR of *PON1* were also investigated in this study, and no significant association was observed. Although these genetic variations have been reported to regulate *PON1* expression¹¹ and contribute to susceptibility to IS,^{23,24} in this study we failed to find any association of them to risk of IS, which should be confirmed by further large-sample studies. For *PON1* rs854571, the results of this study are consistent with previous reports.^{23–25} The *PON2* rs7493 genetic variation causes a substitution (C311S) on exon 9 and has been reported not to be associated with IS risk in Chinese population.^{23–26} Pooled results of published data have also revealed

such an association²⁷ consistent with the results of this study.

In short, this study suggests that *PON1* rs662 is a potential risk of IS, especially for males, and this association is heightened in large-artery atherosclerosis.

Data-Sharing Statement

The data that support the findings of this study are available from the corresponding author Yanping Mei upon reasonable request.

Ethics Statement and Consent

The protocol of this study was in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Nanjing First Hospital, and written informed consent was obtained from all the participants.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Innovation Team of Jiangsu Provincial Health-Strengthening Engineering by Science and Education (CXTDB2017008), Jiangsu Youth Medical Talents Training Project grants to BH (QNRC2016066) and YP (QNRC2016074), and grants from Key Project of Science and Technology Development of Nanjing Medicine (ZKX18030).

Disclosure

The authors report no conflicts of interest for this work.

References

- Johnson W, Onuma O, Owolabi M, Sachdev S. Stroke: a global response is needed. *Bull World Health Organ*. 2016;94(9):634–634A. doi:10.2471/BLT.16.181636
- Price AJ, Wright FL, Green J, et al. Differences in risk factors for 3 types of stroke: UK prospective study and meta-analyses. *Neurology*. 2018;90(4):e298–e306. doi:10.1212/WNL.0000000000004856
- Pastuszek Z, Kozniowska E, Stepień A, Piusinska-Macoch A, Czernicki Z, Koszewski W. Importance rating of risk factors of ischemic stroke in patients over 85 years old in the Polish population. *Neurol Neurochir Pol*. 2018;52(1):88–93. doi:10.1016/j.pjnns.2017.11.007
- Alloubani A, Saleh A, Abdelhafiz I. Hypertension and diabetes mellitus as a predictive risk factors for stroke. *Diabetes Metab Syndr*. 2018.
- Wassertheil-Smoller S, Qi Q, Dave T, et al. Polygenic risk for depression increases risk of ischemic stroke: from the stroke genetics network study. *Stroke*. 2018;49(3):543–548. doi:10.1161/STROKEAHA.117.018857
- Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. *Stroke*. 1993;24(1):35–41. doi:10.1161/01.STR.24.1.35
- Hankey GJ. Stroke. *Lancet*. 2017;389(10069):641–654. doi:10.1016/S0140-6736(16)30962-X
- Humphries SE, Morgan L. Genetic risk factors for stroke and carotid atherosclerosis: insights into pathophysiology from candidate gene approaches. *Lancet Neurol*. 2004;3(4):227–235. doi:10.1016/S1474-4422(04)00708-2
- Gulcher JR, Gretarsdottir S, Helgadottir A, Stefansson K. Genes contributing to risk for common forms of stroke. *Trends Mol Med*. 2005;11(5):217–224. doi:10.1016/j.molmed.2005.03.001
- Deakin SP, Bioletto S, Bochaton-Piallat ML, James RW. HDL-associated paraoxonase-1 can redistribute to cell membranes and influence sensitivity to oxidative stress. *Free Radic Biol Med*. 2011;50(1):102–109. doi:10.1016/j.freeradbiomed.2010.09.002
- Tajbakhsh A, Rezaee M, Rivandi M, Forouzanfar F, Afzaljavan F, Pashdar A. Paraoxonase 1 (PON1) and stroke; the dilemma of genetic variation. *Clin Biochem*. 2017;50(18):1298–1305. doi:10.1016/j.clinbiochem.2017.08.001
- Rodriguez-Esparragon F, Lopez-Fernandez JC, Buset-Rios N, et al. Paraoxonase 1 and 2 gene variants and the ischemic stroke risk in Gran Canaria population: an association study and meta-analysis. *Int J Neurosci*. 2017;127(3):191–198. doi:10.3109/00207454.2016.1165675
- Humbert R, Adler DA, Distèche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet*. 1993;3(1):73–76. doi:10.1038/ng0193-73
- Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. *Br J Pharmacol*. 1997;122(2):265–268. doi:10.1038/sj.bjp.0701390
- Bhattacharyya T, Nicholls SJ, Topol EJ, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA*. 2008;299(11):1265–1276. doi:10.1001/jama.299.11.1265
- Banerjee I. Relationship between Paraoxonase 1 (PON1) gene polymorphisms and susceptibility of stroke: a meta-analysis. *Eur J Epidemiol*. 2010;25(7):449–458.
- Dahabreh IJ, Kitsios GD, Kent DM, Trikalinos TA. Paraoxonase 1 polymorphisms and ischemic stroke risk: A systematic review and meta-analysis. *Genet Med*. 2010;12(10):606–615. doi:10.1097/GIM.0b013e3181ee81e6
- Spychala MS, Honarpisheh P, McCullough LD. Sex differences in neuroinflammation and neuroprotection in ischemic stroke. *J Neurosci Res*. 2017;95(1–2):462–471. doi:10.1002/jnr.23962
- Madsen TE, Khoury JC, Alwell KA, et al. Sex differences in cardiovascular risk profiles of ischemic stroke patients with diabetes in the Greater Cincinnati/Northern Kentucky Stroke Study. *J Diabetes*. 2017.
- Samai AA, Martin-Schild S. Sex differences in predictors of ischemic stroke: current perspectives. *Vasc Health Risk Manag*. 2015;11:427–436.
- Juan J, Jiang X, Tang X, et al. Joint effects of PON1 polymorphisms and vegetable intake on ischemic stroke: a family-based case control study. *Int J Mol Sci*. 2017;18(12):2652. doi:10.3390/ijms18122652
- Cozzi L, Campolo J, Parolini M, et al. Paraoxonase 1 L55M, Q192R and paraoxonase 2 S311C alleles in atherothrombosis. *Mol Cell Biochem*. 2013;374(1–2):233–238. doi:10.1007/s11010-012-1525-2
- Zhang G, Li W, Li Z, et al. Association between paraoxonase gene and stroke in the Han Chinese population. *BMC Med Genet*. 2013;14:16. doi:10.1186/1471-2350-14-16
- Liu ME, Liao YC, Lin RT, et al. A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. *Atherosclerosis*. 2013;228(1):161–167. doi:10.1016/j.atherosclerosis.2013.01.036
- Voetsch B, Benke KS, Panhuysen CI, Damasceno BP, Loscalzo J. The combined effect of paraoxonase promoter and coding region polymorphisms on the risk of arterial ischemic stroke among young adults. *Arch Neurol*. 2004;61(3):351–356. doi:10.1001/archneur.61.3.351
- Xu HW, Yuan N, Zhao Z, et al. Study of the relationship between gene polymorphisms of paraoxonase 2 and stroke in a Chinese population. *Cerebrovasc Dis*. 2008;25(1–2):87–94. doi:10.1159/000111996
- Li BH, Zhang LL, Yin YW, et al. Association between paraoxonase 2 Ser311Cys polymorphism and ischemic stroke risk: a meta-analysis involving 5008 subjects. *Mol Biol Rep*. 2012;39(5):5623–5630. doi:10.1007/s11033-011-1367-0

Pharmacogenomics and Personalized Medicine**Dovepress****Publish your work in this journal**

Pharmacogenomics and Personalized Medicine is an international, peer-reviewed, open access journal characterizing the influence of genotype on pharmacology leading to the development of personalized treatment programs and individualized drug selection for improved safety, efficacy and sustainability. This journal is indexed

on the American Chemical Society's Chemical Abstracts Service (CAS). The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/pharmacogenomics-and-personalized-medicine-journal>