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Lack of association between the Paraoxonase 1 Q/R192 single nucleotide polymorphism and stroke in a Chinese cohort

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Abstract

Background: Serum paraoxonase (PON1) is an HDL-associated esterase that hydrolyzes products of lipid peroxidation and prevents the oxidation of LDL. Paraoxonase 1 (PON1) was implicated in susceptibility to stroke in previous studies. We investigated the correlation between the paraoxonase Gln-Arg 192 polymorphism (PON1Q/R192) and stroke including cerebral hemorrhage and cerebral infarction.

Method: The association between the paraoxonase Gln-Arg 192 polymorphism (PON1Q/R192) and stroke was investigated in 1019 subjects, which involved 305 cases with cerebral hemorrhage, 375 cases with cerebral infarction and 339 healthy controls.

Results: The PON1Q/R192 genotype distribution in the cerebral hemorrhage group was QQ13.1%, QR48.2% and RR38.7% and in the cerebral infarction group was QQ13.6%, QR44.0% and RR42.4% respectively. There was no significant difference in PON1Q/R192 allele and genotype distribution between the patient group and the control group ($P > 0.05$). The PON1 polymorphism was not associated with cerebral hemorrhage or infarction.

Conclusion: Our study suggests that serum paraoxonase (PON1) is not associated with cerebral hemorrhage or infarction, although it is a lipolactonase which is associated with HDL-apolipoprotein A-I (HDL-apoA-I) and plays a role in the prevention of atherosclerosis.

Key words: Paraoxonase 1; cerebral hemorrhage; stroke; ischemic stroke; polymorphism.

Introduction

Serum paraoxonase (PON1) is a HDL-associated enzyme, which belongs to a family of calcium-dependent hydrolases (Primo-Parmo SL, 1996). It can prevent the oxidation of low-density lipoprotein (LDL) cholesterol which is believed to play an important role in the initiation of atherosclerosis. Serum PON1 levels seem to be inversely related to the level of cardiovascular disease (Ayub, 1999; Mackness, 2003; Navab, 1997). Despite the evidence that PON1 prevents atherosclerosis in animal models (Shih, 2000; Shih, 1998; Tward, 2002; Rozenberg, 2003), it remains to be established whether PON1 possesses atheroprotective and antioxidant properties in humans (Bhattacharyya, 2008). Several studies have suggested that PON1 may play an atheroprotective role, but the simultaneous associations between PON1 polymorphisms and enzyme activity with cerebrovascular disease (CVD) risk have been reported in only some population studies (Mackness and Mackness, 2004; Li, 2003; Durrington, 2001; Hegele, 1999; Voetsch, 2002; Yamada, 2002).

The PON1 gene has two common coding-region polymorphisms, which regulate the concentration and activity of the enzyme and alter its ability to prevent lipid oxidation. Although a meta-analysis concluded that the *PON1-192* R allele was weakly but significantly associated with an increased risk of atherosclerosis, other studies, reported a trend towards increased atherosclerosis risk in individuals with the QQ genotype (Aubó, 2002; Zuliani, 2002; Turban, 2001).

Cerebral hemorrhage is a life-threatening condition which is associated with substantial morbidity and mortality. Hypertension is an important risk factor of cerebral hemorrhage and atherosclerosis, whereas atherosclerosis is one of the most important risk factors leading to cerebral infarction. There have been many case-control studies to test the hypothesis, that the 192R allele of the PON1 gene is associated with coronary heart disease (CHD) (Durrington, 2001). But there is less evidence elucidating the association between 192R allele of the PON1 gene and cerebral infarction as well as intracerebral hemorrhage (ICH).

The purpose of this study was to assess the distribution of polymorphisms of the *PON1* genes in well-phenotyped patients with stroke and matched control subjects, and to find the correlation between the PON1Q/R192 and stroke in these samples.

Subjects and Method

1.1. Subjects

Between February 2001 and November 2002, total of 680 survived patients with stroke, comprised of 305 patients with cerebral hemorrhage and 375 patients with cerebral infarction, with an average age of 60.8 ± 11.4 years were referred to the Second Xiangya Hospital of Central-South University, in Changsha, China. All the patients were investigated within 24h after occurrence and all the subjects enrolled in the study were Chinese Hans. The diagnosis of stroke was based on findings of history and physical examination and confirmed by CT and/or MRI of the brain. The control population was 339 apparently healthy people without cardiocerebrovascular diseases, from an outpatient department or physical check-up department, simultaneously.

Subjects with following incorporated diseases were excluded as hematopathy, tuberculosis, malignant tumor, hepatic or renal failure, the use of drugs, eg: anticoagulants, or persons with lipid-lowering pharmacological therapy prior to hospital admission less than one week. The study was approved by the Hospital Ethics Committee and informed consent was obtained from all subjects or their relatives.

1.2. Research method

1.2.1. *Determination of PON1Q/R192 genotype*

Peripheral blood samples were collected after study subjects' consent. Genomic DNA was isolated from white blood cells using standard phenol-chloroform extraction. Genotypes were determined by polymerase chain reaction (PCR) and restriction enzyme digestion. For the PON1 192 polymorphism, forward primer 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and reverse primer 5'- CAC GCT AAA CCC AAA TAC ATC TC-3' were used (Mackness B, 1998). The amplification cycle for PON1 polymorphism genotyping was performed in a Gene Amp PCR system 2400 with an initial 3-min denaturation period at 94°C, followed by 32 cycles of 40s at 94°C, 50s at 58°C, and 40s at 72°C, with a final extension of 10 min at 72°C. Digestion of the PCR production with 3U of BspPI resulted in three fragments of 99bp, 66bp and 33bp. All digested products were analyzed on 3% agarose gel stained with ethidium bromide and examined under ultraviolet transillumination.

1.2.2. *Statistical analysis*

Ver.13.0 of the Statistical Package for Social Sciences (SPSS) for Windows was used for all statistical analyses. Student's two-tailed t test for independent samples, $\chi 2$ test, or One-way ANOVA was used to assess the differences between the groups when appropriate. A conditional logistic regression model was used to calculate odds ratios (ORs) with 95% CIs for the effect of the risk factors to the early development of clinically manifested cerebral hemorrhage. Data are presented as a geometric mean ± SEM or percentages.

Results

2.1. General characteristics in stroke cases and randomly selected controls

Table 1 shows age, sex, body mass index, risk factors and alcohol measurements in case groups and control group. There was no significant difference among the three groups with respect to age, body mass index and alcohol measurements. The frequency of some risk factors involved in stroke such as history of hypertension, diabetes mellitus, systolic pressure, diastolic pressure, family history with stroke and smoking were significantly higher in the two stroke groups than in control group ($P < 0.05$) (Table 1).

2.2. Distribution of genotype and allele frequencies of the PON1-192 gene in the Case group and control group

The distribution of genotype and allele frequencies of Q192R gene polymorphism between case and control groups was not significantly different.

The distribution of the PON1 192 genotypes was in Hardy-Weinberg equilibrium, both in the Cerebral infarction- controls subjects ($\chi 2 = 1.18$, $df = 1$, $p > 0.5$) and Cerebral hemorrhage - controls subjects ($\chi^2 = 0.26$, $df = 1$, $P > 0.5$) (Table 2). Expected genotype frequencies were calculated by the Hardy-Weinberg equation from the allele frequencies. All these differences failed to reach statistical significance ($P > 0.05$).

2.3. Analysis of risk factors for stroke

A conditional logistic regression model was used to calculate odds ratios (ORs) with 95% CIs for the

Table 1

Demographic and clinical characteristics of Patients with Cerebral Infarction and Controls

Variables	Control (n = 339)	Cerebral infarction patient (n = 375)	Cerebral hemorrhage patient (n = 305)
Mean age (mean ± SD, year)	61.6 ± 7.1	62.7 ± 11.3	60.8 ± 11.4
Sex (M/F)	191/148	222/153	193/112
BMI (kg/m^2)	23.4 ± 3.5	23.8 ± 3.4	23.6 ± 4.1
Systolic pressure (mmHg)	139.0 ± 21.6	159.3 ± 29.0	162.3 ± 29.2
Diastolic pressure (mmHg)	81.1 ± 10.5	92.9 ± 16.0	97.5 ± 18.0
Family history of stroke	4.4%	21.1%	28.9%
History of hypertension	38.6%	68.3%	73.8%
History of diabetes	6.2%	19.7%	14.4%
Current smoking	39.8%	49.3%	49.2%
Current drinking	24.5%	28.5%	30.5%

Table 2

Genotype distribution and allelic frequencies of the PON1 gene in CI patient and control group

Group	Control (n = 339)	Cerebral infarction patient (n = 375)	Cerebral hemorrhage patient (n = 305)
Genotype (%)			NS
QQ	55 (16.2)	42 (11.2)	40 (13.1)
QR	150 (44.3)	164 (43.7)	147 (48.2)
RR	134 (39.5)	169 (45.1)	118 (38.7)
Allele frequencies (%)			
Q	38.35	33.07	37.21
R	61.65	66.93	62.83

effect of the risk factors to the early development of clinically manifested cerebral hemorrhage. The QQ genotype yielded an OR of 0.779 (95% CI, 0.502 to 1.211) ,while the RR genotype yielded an OR of 0.965 (95% CI, 0.703-1.325) by univariate analysis. The distribution of the PON1 192 genotypes and alleles did not differ significantly between the two groups.

The same model was used to calculate odds ratios (ORs) with 95% CIs for the effect of the risk factors to the early development of clinically manifested CI. The QQ genotype yielded an OR of 0.951 (95% CI, 0.423 to 1.003), while the RR genotype yielded an OR of 1.255 (95% CI, 0.932-1.691) by univariate analysis. The distribution of the PON1 192 genotypes and alleles did not differ significantly between the two groups.

Discussion

Over the past ten years, several studies have suggested that PON1 is implicated in the pathogenesis of atherosclerosis, coronary heart disease, and stroke (Oda, 2002; Tward, 2002). Many factors (for example hypertension) affect the development of atherosclerosis, but these factors and the importance of each differ between cerebral and coronary arteries. Clinical Research data suggest that PON1 protects LDL from peroxidation in the intima and plays an important role in the protection of the development of atherosclerosis. But there exists a difference in the constitution and environment between coronary and cerebral arteries. The protective effect of the PON1 gene against atherosclerosis seems weaker in the cerebral than in the coronary arteries. Our research is the first report to have examined the effect of paraoxonase (PON1) Q192R polymorphism in cerebral hemorrhage. And the data shows no association between *PON1* Q192R polymorphism and stroke, suggesting that the *PON1 Q192R* polymorphism and atherosclerosis are not definitive risk factors for cerebral infarction or hemorrhage in elderly individuals in china.

Stroke is a multifactorial disease controlled by genetic and environmental factors (Chai, 2008; Komori, 2008; Graff-Iversen, 2007). Twin and family studies have determined that there is a significant familial or genetic component underlying the occurrence of stroke. In our study, history of hypertension, diabetes mellitus, systolic blood pressure, diastolic blood pressure, family history with stroke and smoking may be the risk factors for stroke ($P < 0.05$),whereas among the case groups and control group, there were no significant

differences in age, body mass index and alcohol measurements.

In a recent meta-analysis of several studies, one of which involved 350 cases (Shin, 2008), the studies elucidated a significant difference between the apolipoprotein (Apo) B concentration and the PON1Q192R polymorphism (P = 0.02) but showed no significant overall associations with ischemic stroke. The similar result was reported by Schiavon (Schiavon, 2007) whose study showed similar genotype distribution for PON1 Q192R polymorphism in acute ischemic stroke patients and healthy subjects. While there have been fewer studies in ischemic stroke, the evidence shows the prevalence of the PON1 192RR genotype (P = 0.006) and significant increase frequency of the R allele (P = 0.010) among young acute ischemic stroke patients compared with controls (Voetsch, 2002). This differs from a study (Can Demirdöğen, 2008), not included in the meta-analysis, which suggested that PON1 activity ratio, PON1 192RR genotype and PON1 status as an important risk factors for ischemic stroke. In contrast to this study, our results are not consistent with the previous report. The possible explanation why our results did not support the positive association between PON1 and ischemic stroke was that the association may be different among the sample populations with different ethnic backgrounds. In the previous study, the patients were selected from the Turk population, whereas all our samples were obtained from Chinese individuals.

The PON1-Q192R polymorphism also displayed a significant interracial distribution as described by former reports. The frequencies of the Q/R allele were 0.6–0.7/0.3–0.4 in the White and 0.3–0.4/0.6–0.7 (Imai, 2000; Topić, 2001)in the Asian Chinese and Japanese. Allele frequencies were 0.69 and 0.31 for Q and R genetic variants respectively in Turkish population, whose country lies at the junction between Europe and Asia (Aynacioglu, 1999). Allele frequencies also have an association with geographical location in the same population (Allebrandt, 2002). We investigated the polymorphism of PON1 Glen 192 Arg (A G) genotypes using a PCR-RFLP technique, and found that QR genotype (44.0%) was predominant in the total subjects. In our cerebral infarction studies, the distribution tendency of Q/R alleles in Chinese Han population of Hunan was 0.366/0.634, which is consistent with the previous results of Asian Chinese and Japanese. Allele frequency of R was slightly higher than those reported in Chinese Hans (0.52 and 0.55).

In conclusion, no significant difference of genotype and allelic distribution in both PON1-Q192R polymorphisms in stroke patients and controls was revealed, neither significant difference was observed in lipid profiles among the three genotypes. Further studies are necessary to expand the sample size and investigate the definite result.

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REFERENCES

Allebrandt KV, Souza RL, Chautard-Freire-Maia EA. Variability of the paraoxonase gene (PON1) in Euro- and Afro-Brazilians. Toxicol Appl Pharmacol. 2002;180:151-156.

Aubó C, Sentí M, Marrugat J, Tomas M, Vila J. *et al.* Risk of myocardial infarction associated with Gln/Arg 192 polymorphism in the human paraoxonase gene and diabetes mellitus. The REGICOR Investigators. Eur Heart J. 2000;21:33-8.

Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Nacak M, Tapanyigit EE. *et al.* Paraoxonase 1 mutations in a Turkish population. Toxicol Appl Pharmacol. 1999; 157: 174-177.

Ayub A, Mackness MI, Arrol S, Mackness B, Patel J. *et al.* Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol. 1999;19(2):330-5.

Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X. *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.

Can Demirdöğen B, Türkanoğlu A, Bek S, Sanisoğlu Y, Demirkaya S. *et al.* Paraoxonase/arylesterase ratio, PON1 192Q/R polymorphism and PON1 status are associated with increased risk of ischemic stroke. Clin Biochem. 2008;41(1-2):1-9.

Chai J, Chu FC, Chow TW, Shum NC. Prevalence of malnutrition and its risk factors in stroke patients residing in an infirmary. Singapore Med J. 2008; 49(4):290-6.

Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21(4):473-480.

Graff-Iversen S, Jenum AK, Grøtvedt L, Bakken B, Selmer RM, *et al.* Risk factors for myocardial infarction, stroke and diabetes in Norway. Tidsskr Nor Laegeforen. 2007;127(19):2537-41.

Hegele RA. Paraoxonase genes and disease. Ann Med. 1999;31(3):217-224.

Imai Y, Morita H, Kurihara H, Sugiyama T, Kato N. *et al.* Evidence for association between paraoxonase gene polymorphisms and atherosclerotic disease. Atherosclerosis. 2000;149:435-442.

Komori T, Eguchi K, Tomizawa H, Ishikawa J, Hoshide S. *et al.* Factors associated with incident ischemic stroke in hospitalized heart failure patients: a pilot study. Hypertens Res. 2008;31(2):289-94.

Li HL, Liu DP, Liang CC. Paraoxonase gene polymorphisms, oxidative stress, and diseases. J Mol Med. 2003;81(12):766-779.

Mackness B, Durrington P, McElduff P, Yarnell J, Azam N. *et al.* Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation. 2003;107(22):2775-9.

Mackness B, Mackness MI, Arrol S, Turkie W, Julier K. *et al.* Serum paraoxonase 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis. 1998;139(2):341-9.

Mackness M, Mackness B. Paraoxonase 1 and atherosclerosis: is the gene or the protein more important? Free Radic Biol Med. 2004;37(9):1317-1323.

Navab M, Hama-Levy S, Van Lenten BJ, Fonarow GC, Cardinez CJ. *et al.* Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. J Clin Invest. 1997; 99(8):2005-19.

Oda MN, Bielicki JK, Ho TT, Berger T, Rubin EM. *et al.* Paraoxonase 1 overexpression in mice and its effect on high-density lipoproteins. Biochim Biophys Res Commun. 2002; 290:921-927.

Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics. 1996;33(3):498-507.

Rozenberg O, Rosenblat M, Coleman R, Shih DM. *et al.* Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. Free Radic Biol Med. 2003;34(6):774-784.

Schiavon R, Turazzini M, De Fanti E, Battaglia P, Targa L. *et al.* PON1 activity and genotype in patients with arterial ischemic stroke and in healthy individuals. Acta Neurol Scand. 2007;116(1):26-30.

Shin BS, Oh SY, Kim YS, Kim KW. The paraoxonase gene polymorphism in stroke patients and lipid profile. Acta Neurol Scand. 2008;117(4):237-43.

Shih DM, Gu L, Xia YR, Navab M, Li WF. *et al.* Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature. 1998;394(6690):284-287.

Shih DM, Xia YR, Wang XP, Miller E, Castellani LW. *et al.* Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. J Biol Chem. 2000;275(23):17527-35.

Topić, E, Simundić AM, Ttefanović M, Demarin V, Vuković V. *et al.* Polymorphism of apoprotein E (APOE), methylenetetrahydrofolate (MTHFR) and poraoxonase (PON1) genes in patients with cerebrovascular disease. Clin Chem Lab Med. 2001;39:346-350.

Turban S, Fuentes F, Ferlic L, Brugada R, Gotto AM. *et al.* A prospective study of paraoxonase gene Q/R192 polymorphism and severity, progression and regression of coronary atherosclerosis, plasma lipid levels, clinical events and response to fluvastatin. Atherosclerosis. 2001;154:633-40.

Tward A, Xia YR, Wang XP, Shi YS, Park C. *et al.* Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation. 2002;106(4):484-490.

Voetsch B, Benke KS, Damasceno BP, Siqueira LH, Loscalzo J. Paraoxonase 192 Gln —> Arg polymorphism: an independent risk factor for nonfatal arterial ischemic stroke among young adults. Stroke. 2002;33(6):1459-64.

Yamada M, Sodeyama N, Itoh Y, Otomo E, Matsushita M. *et al.* No association of paraoxonase genotype or atherosclerosis with cerebral amyloid angiopathy. Stroke. 2002;33(4):896-900.

Zuliani G, Cherubini A, Volpato S, Palmieri E, Mecocci P. *et al.* Genetic factors associated with the absence of atherosclerosis in octogenarians. J Gerontol A Biol Sci Med Sci. 2002;57:M611-5.

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