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ORIGINAL ARTICLES

Association studies of genetic polymorphism and environmental factors in ischemic stroke with atherosclerotic middle cerebral artery stenosis

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Abstract

Objectives: Intracranial atherosclerosis, especially the middle cerebral artery (MCA), is the commonest vascular lesion for ischemic stroke the Chinese population. We explored the association of genetic polymorphism and environmental factors in MCA atherosclerosis in the Chinese population. *Methods:* One hundred fifty-six ischemic stroke patients with MCA stenosis and 181 well-matched ischemic stroke patients without MCA stenosis were examined by polymerase chain reaction (PCR). The PCR products were analyzed for lipoprotein lipase (LPL) S447X and paraoxonase1 (PON1) Q192R polymorphisms by restriction enzyme digestion. Medical history documentation and investigation of biochemical markers were performed for each subject. *Results:* Univariate analysis showed that the levels of systolic blood pressure (SBP) were higher in the MCA stenosis group. There were no significant differences in the genotype and allele frequencies of the LPL S447X and PON1 Q192R polymorphism observed between the two groups. But, in the patients above 60 years of age with and without MCA stenosis, LPL X carriers have higher level of SBP than the LPL SS genotype carriers. Multivariate logistic regression found that SBP was the significant, independent predictor of the presence of MCA stenosis patients above 60 years of age ($P < 0.001$, OR=1.206, 95% confidence intervals: 1.014-1.032).
Conclusions: SBP appears to contribute to the pathogenesis of MCA stenosis among Chinese. The gene polymorphism of LPL S447X may be associated with atherosclerotic MCA stenosis in Chinese population.

Keywords: Ischemic stroke, lipoprotein lipase gene, middle cerebral artery, paraoxonase1gene.

INTRODUCTION

Intracranial atherosclerotic disease is one of the most common causes of ischemic stroke worldwide. Intracranial artery occlusive diseases, especially middle cerebral artery (MCA) stenosis, are major causes of ischemic stroke in Asian, Black, and Hispanic populations.[1] In Chinese populations, MCA stenosis account for approximately 33-50% of stroke and 45% of transient ischemic attack.[2]

Ischemic stroke is a multifactorial disease. In addition to conventional risk factors such as hypertension, diabetes mellitus (DM), hyperlipidemia, and smoking. Evidence from twin, case-control and cohort studies of familial have repeatedly indicated a genetic contribution to ischemic stroke susceptibility.[3,4] Genetic risk factors are often considered not to be modifiable; however, understanding these genetic influences may lead to better prevention of and intervention in stroke. Therefore, the genome wide association studies (GWAS) have identified a number of

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loci which contribute to plasma lipid levels, the identification of 95 loci for blood lipids have been reported in a collaborative GWAS study involving over 100,000 probands.[5]

Lipoprotein lipase (LPL) and paraoxonase1 (PON1) are key enzymes in lipid and lipoprotein metabolism and variants in its gene have been associated with lipid levels in all the GWAS studies cited above. The LPL gene is located on chromosome 8p22, spans approximately 35 kb and encoding a 448 amino acid mature protein.[6] LPL is a rate-limiting enzyme responsible for hydrolysis of plasma triglycerides (TG) and converts very-low-density lipoprotein (VLDL) to low-density lipoprotein (LDL). The S447X polymorphism (rs328) has been shown that the stability and catalytic activity in the truncated form is normal, is associated with increased LPL protein secretion and plasma post-heparin activity, as well as decreased plasma TG levels and increased high-density lipoprotein (HDL) levels.[7] But, the relationship between LPL S447X polymorphism and vascular disease remains controversial.[8-10]

The human PON1 gene spans approximately 26 kb and is located on chromosome 7q21-22.[11] PON1 is synthesized in liver, secreted to the blood and combines with HDL, and is one of the most studied gene regarding cardiovascular risk, oxidative stress and inflammation.[12,13] PON1 has been shown to prevent LDL oxidation, in addition, there is evidence from animal and in vitro models that PON1 can protect the HDL particle from oxidation and preserve the integrity of HDL.[14] This gene possesses several polymorphisms and among them the most investigated is PON1 Q192R (rs662). Some studies found that both the PON1 Q192R genotype and the level of plasma PON1 are a powerful predictor for vascular disease,[15,16] and suggested the 192R allele might increase the risk of coronary heart disease or ischemic stroke.[17, 18] But, other reports finding no relationship between R allele and vascular disease.[19] Therefore, the aim of this study was to analyze the association between Polymorphisms of these genes with MCA atherosclerotic stenosis in Chinese stroke patients. These results may be helpful for predicting the development of atherosclerotic MCA stenosis and for defining appropriate strategies for decreasing mortality through early-stage intervention.

METHODS

Patients and techniques

This case-control study included ischemic stroke patients with MCA stenosis and without MCA stenosis. All ischemic stroke patients who were admitted to the Acute Stroke Unit of the Prince of Prince of Wales Hospital consecutively over a period of nine months. All patients were of Chinese origin. Age, sex, lipid concentrations, and vascular risk factors, including hypertension, DM, smoking habit and history of ischemic heart disease were collected from controls or during acute admission for patients. Hypertension was defined as systolic blood pressure (SBP)>140 mmHg or diastolic blood pressure (DBP) >90 mmHg on at least two occasions, or treatment with blood pressure-lowering medications. DM was defined as fasting plasma glucose >7.0 mmol/L. TG, total cholesterol (TC), and HDL were measured, and LDL calculated using Friedewald's formula. The Research Ethics Committee approved this study, and all participants signed informed consent form, according to the Helsinki Declaration.

The degree of stenosis was determined by magnetic resonance angiography (MRA) or transcranial doppler ultrasonography (TCD). MCA stenosis on MRA was defined as more than 50% lumen diameter reduction. The criteria of TCD for MCA stenosis were defined by the peak systolic flow velocity of equal to or more than 140cm/s. Patients with nonatherosclerotic causes of vascular stenosis (eg, dissection, moyamoya disease, vasculitis) were excluded.

Genotyping

Polymerase chain reaction (PCR)-based protocols were used to identify the genotype and allele frequencies of two gene polymorphisms, in the LPL gene and PON1 gene. Genomic deoxyribonucleic acid (DNA) extracted from peripheral blood was amplified by PCR. The genotyping of LPL was described in detail previously.[20]

PCR of PON1 used the following components: 400 mM dNTP, 1 unit TaqGold (Applera), 1X TaqGold Buffer, 250 nM forward primer TATTGTTGCTGTGGGACCTGAG, 250 nM reverse primer CACGCTAAACCCAAATACATCTC, 2.5 mM $MgCl_2$, 1 ml DNA, and water to a total volume of 20 ml. PCR was performed at 95°C for 10 min followed by 40 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min, followed by 72°C for 10 min and storage at 4°C. Products were digested overnight at 37°C with 2.5 U AlwI (New England BioLabs) in 1X Buffer 4 and resolved on 12% polyacrylamide gels. Q192 alleles remained uncut at 99 bp, while R192 alleles were cut.

Statistical methods

Statistical analyses were performed using SPSS13.0 (Statistical Package for the Social Sciences 13.0), with significance set at $P < 0.05$. A chi-square goodness-of-fit test was used to test the distribution of genotypes and allele frequencies for deviations from the Hardy–Weinberg equilibrium. Differences in the genotype and allele frequencies between the subjects with or without MCA stenosis were analysed using chi-square test and odds radios with Cornfield 95% confidence intervals. Data from normally distributed parameters are presented as mean ± SD. Student's t-test was used to seek any differences between the subjects with and without MCA stenosis, for the variables such as age, SBP, DBP, TC, HDL, LDL and TG. Any the above variables that were significantly associated with MCA stenosis on univariate analysis, including genotypes of the LPL and PON1 genes, were included in the multivariate logistic regression model.

RESULTS

Patient characteristics

We included a total of 390 acute ischemic stroke patients during our study period. Twenty-five cases were excluded as no MRA or TCD study was performed on them, while another 28 cases were excluded due to poor temporal window on TCD and MRI was not performed on them. Thus, 337 ischemic stroke patients were recruited. Among the recruited cases, MCA stenosis was detected in 156 subjects (14 subjects under 60 years of age and 142 subjects above 60 years of age), while the other 181 had no MCA stenosis (33 subjects under 60 years of age and 148 subjects above 60 years of age).

There were no significant differences in terms of demographic features and lipid levels between the two groups. But, univariate analysis (Table 1) showed that the level of SBP was higher in the MCA stenosis group (P = 0.000). There were trends toward MCA stenosis patients having higher level of DBP (P = 0.058), more male (P = 0.072).

Gene polymorphism and allele distributions

Genotype frequencies in all groups were in Hardy-Weinberg equilibrium (All $P > 0.05$). There were no significant differences in the genotype and allele frequencies of the LPL and PON1 polymorphism observed between patients with and without MCA stenosis (Table 2). But, there was a trend that the MCA stenosis group tended to have more of genotype LPL XX (4.5% vs 1.1%) than the non-MCA-stenosis group (P = 0.087). However, there were no significant differences in the genotype and allele frequencies of the LPL and PON1 polymorphism observed between patients (subjects above 60 years of age) with and without MCA stenosis (Table 3).

Table 1: Demographic features and prevalence rates of risk factors in the ischemic stroke patients with and without MCA stenosis

Measure	MCA-stenosis (N = 156)	Non-MCA-stenosis (N = 181)	p Value
Age	72.46±10.13	70.39±12.45	0.100[b]
Gender(male)	86 (55.1 %)	82 (45.3%)	0.072[a]
Hypertension	101 (64.7 %)	121 (66.9 %)	0.684[a]
DM	45 (28.8.3 %)	59 (32.6 %)	0.457[a]
Ischemic heart disease	16 (10.3 %)	19 (10.5 %)	0.942[a]
Hyperlipidemia	97 (62.2 %)	98 (54.1 %)	0.136[a]
Smoker	65 (41.7 %)	70 (38.7 %)	0.576[a]
SBP	175.94±28.27	160.56±26.06	0.000[b]
DBP	86.49±16.80	82.81±16.78	0.058[b]
TC	5.19±1.23	5.06±1.24	0.280[b]
HDL	1.39±0.35	1.38±0.37	0.910[b]
LDL	3.14±1.04	2.95±0.97	0.104[b]
TG	1.56±0.80	1.53±0.88	0.796[b]

[a]chi-square test; [b]Student's t-test; MCA, middle cerebral artery; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high density lipoprotein; LDL, low-density lipoprotein; TG, Triglyceride.

Table 2: LPL and PON1 gene polymorphism and allele distributions in the ischemic stroke patients with and without MCA stenosis

Measure	MCA-stenosis (N = 156)	Non-MCA-stenosis (N = 181)	p Value
LPL genotypes			
SS	118 (75.6 %)	143 (79.0 %)	0.461[a]
SX	31 (19.9 %)	36 (19.9 %)	0.997[a]
XX	7 (4.5 %)	2 (1.1 %)	0.087[a]
LPL alleles			
S	267 (85.6 %)	322 (89.0 %)	0.188[a]
X	45 (14.4 %)	40 (11.0 %)	0.188[a]
PON1 genotypes			
RR	51 (32.7 %)	60 (33.1%)	0.929[a]
RQ	86 (55.1 %)	100 (55.2 %)	0.982[a]
QQ	19 (12.2 %)	21 (11.6 %)	0.870[a]
PON1 alleles			
R	188 (60.3 %)	220 (60.8 %)	0.891[a]
Q	124 (39.7 %)	142 (39.2 %)	0.891[a]

p-values are for chi-square test or Fisher's exact test; MCA, middle cerebral artery; LPL, Lipoprotein lipase; PON1, paraoxonase 1.

Association of genotypes with risk factors

Among the patients with and without MCA stenosis, compared with LPL SS genotypes, LPL X carrier group were not significantly associated with SBP, DBP, TC, HDL, LDL and TG. But, in the patients above 60 years of age with MCA stenosis, LPL X carriers have higher level of SBP than the LPL SS genotype carriers (Table 4).

Multivariate logistic regression found that SBP was the significant, independent predictor of the presence of MCA stenosis patients above 60 years of age ($P < 0.001$, OR=1.206, 95% confidence intervals: 1.014-1.032).

Table 3: LPL and PON1 gene polymorphism and allele distributions in the ischemic stroke patients with and without MCA stenosis (above 60 years of age)

Measure	MCA-stenosis (N = 142)	Non-MCA-stenosis (N = 148)	p Value
LPL genotypes			
SS	108 (76.1 %)	120 (81.1 %)	0.297[a]
SX	29 (20.4 %)	27 (18.2 %)	0.638[a]
XX	5 (3.5 %)	1 (0.7 %)	0.114[a]
LPL alleles			
S	245 (86.3%)	267 (90.2 %)	0.141[a]
X	39 (13.7%)	29 (9.8 %)	0.141[a]
PON1 genotypes			
RR	46 (32.4 %)	45 (30.4 %)	0.715[a]
RQ	77 (54.2 %)	88 (59.5 %)	0.368[a]
QQ	19 (12.2 %)	15 (10.1 %)	0.390[a]
PON1 alleles			
R	169 (59.5 %)	178 (60.1 %)	0.877[a]
Q	115 (40.5%)	118 (39.9 %)	0.877[a]

p-values are for chi-square test or Fisher's exact test; MCA, middle cerebral artery; LPL, Lipoprotein lipase; PON1, paraoxonase 1.

Table 4: Association of LPL different genotypes with risk factors in the ischemic stroke patients with and without MCA stenosis (above 60 years of age)

Measure	MCA-stenosis		p Value	Non-MCA-stenosis		p Value
	SX+XX (N = 34)	SS (N = 108)		SX+XX (N = 28)	SS (N = 120)	
SBP	188.30±26.37	174.60±27.52	0.042	153.48±19.93	160.36±26.48	0.211
DBP	89.90±14.18	85.33±16.67	0.177	77.44±13.98	81.25±15.68	0.250
TC	5.27±1.11	5.16±1.15	0.623	4.87±1.16	5.00±1.21	0.587
HDL	1.46±0.35	1.35±0.36	0.093	1.39±0.35	1.39±0.38	0.553
LDL	3.17±0.89	3.12±1.11	0.805	2.93±0.95	2.92±0.99	0.984
TG	1.43±0.82	1.63±0.83	0.224	1.49±0.82	1.45±0.69	0.718

MCA, middle cerebral artery; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high density lipoprotein; LDL, low-density lipoprotein; TG, Triglyceride; LPL, Lipoprotein lipase.

DISCUSSION

To our knowledge, this is the first study evaluating the association between the polymorphism of LPL and PON1 genes with MCA atherosclerotic stenosis. Patients with nonatherosclerotic causes of vascular stenosis were excluded. The degree of MCA stenosis was confirmed with TCD and MRA.

In our study, we have found that SBP was the only independent predictor of MCA stenosis among Chinese ischemic stroke patients, which was consistent with the finding of other studies among stroke free Chinese diabetic patients with or without MCA stenosis or ischemic stroke occurrence in the Greek population.[21,22] In another study among Japanese stroke patients comparing clinical features of those with intracranial large arteries and cervical carotid artery diseases also showed that hypertension was associated with the former, while hyperlipidemia was associated with the later.[23] Tuladhar *et al*.[24] found that hyperlipidemia was associated with carotid stenosis, but no association with MCA stenosis in ischemic stroke patients. Even among intracranial arteries, some investigators have suggested that risk factors for vertebral artery and basilar artery lesions differ from those for MCA lesions.[25,26] Hyperlipidemia and ischemic heart disease seem to play an important role in extracranial internal carotid artery and basilar artery lesions, while MCA stenosis is closely related to hypertension.[26]

So far, little data are available for other genes associated with atherosclerosis especially among ischemic stroke patients with MCA stenosis. Considering the effects of polymorphisms in two genes, no significant differences were found for LPL and PON1 between MCA stenosis and non MCA stenosis groups. But, there was a trend that the MCA stenosis group tended to have more of genotype LPL XX (4.5% vs 1.1%, p=0.087) than the non-MCA-stenosis group, and the frequency of X allele 14.4%, with no differences between non-MCA-stenosis group (11.0%), which was consistent with the finding of other studies among community and acute myocardial infarction patients.[9,10] But, some studies found that the X allele was associated with reduced risk of vascular disease.[8,27] I think there are several possibilities can be inferred here. First, LPL gene S447X variant are of small effect in the pathogenesis of atherosclerosis. Second, the S447X variant might interact with other genetic variants in or near LPL gene or environment determinants such as hypertension, DM and smoking. Third, being cross-sectional with a small sample size was a major limiting factor in assessing the study hypothesis. We agree that confirmation of our results in a large prospective, well-designed study is critical.

We also found that LPL X carrier group were not significantly associated with blood pressure and blood lipid levels in two groups. Some studies suggested an association between the 447X variant with decreased blood pressure[28,29], but other studies found no association at all.[30] Wittrup *et al*.[31] found that the LPL S447X polymorphisms did not cause an increase in the plasma TG concentration. But, the research indicated that the levels of TG in 447XX carriers are significantly lower than that in SS carriers and the TC in the same genotype was significantly lower than that in non-carriers.[9] Similar studies indicate that the X447 allele is associated with lower plasma TG, TC and LDL levels, higher levels of HDL.[32,33] Now, The relationship among LPL S447X

polymorphism with blood pressure and blood lipid levels remains controversial. Further larger studies and in different ethnic are needed to explore the potential role of LPL in MCA stenosis.

In conclusion, the main finding from the present study is that only SBP is a major risk factor of MCA stenosis in the ischemic stroke Chinese patients. The gene polymorphism of LPL S447X and PON1 Q192R were not associated with atherosclerotic MCA stenosis in Chinese population but larger studies are needed to confirm this observation.

DISCLOSURE

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