Original Article
Relationship between lipoprotein lipase gene polymorphism and hemorrhagic stroke in a Chinese population

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Abstract: Objective: To investigate the relationship between lipoprotein lipase (LPL) gene polymorphism and cerebral hemorrhage in a Chinese population. Method: This study was based on the case-control study, PCR-RELP and sequencing method were utilized for genotyping. LPL gene Hind III polymorphism was detected both in 300 patients with cerebral hemorrhage (CH group) and in 300 healthy control subjects (control group). Blood lipid level and blood glucose were detected at the same time. Result: Our results showed that G allele frequency was significant lower in the CH group than that in the control group (OR=0.611; 95% CI: 0.427-0.876, P=0.001). We also found both GG (OR=0.543, 95% CI: 0.233-0.988; P=0.041) and TG (OR=0.609, 95% CI: 0.387-0.959, P=0.032) genotype were frequent in the control group than that in the CH group. TG level of the groups who carry TT genotype were much higher than that of the groups carrying TG+GG genotype (P<0.05). By means of adjusting age, hypertension and hyperglycemia, logistic multivariate regression analysis revealed that LPL Hind III G allele might be a protective factor (OR=0.601, 95% CI: 0.231-0.876; P=0.001) in the present study. Conclusion: It is suggested that LPL Hind III G allelic mutation might be a protective factor against cerebral hemorrhage disease in Chinese population.

Keywords: Cerebral hemorrhage, lipoprotein lipase, PCR, gene frequency, genotype, risk factors

Introduction

Lipoprotein lipase (LPL) plays a key role in the hydrolysis of circulating triglyceride-rich lipoproteins, such as chylomicrons and VLDL [1-3]. LPL is the predominant plasma triglyceridelipase and is bound to vascular endothelium through interaction with membrane-anchored proteoglycans. Previous studies showed that abnormal lipoprotein lipase was closely related to occurrence of hyperlipidemia [4], atherosclerosis [5] stroke [6], diabetes mellitus [7], chronic kidney disease [8] and tumors [9]. Cerebral hemorrhage is a common type of hemorrhagic stroke. Some studies have indicated that individual genetic diversity also correlates with the risk of cerebral hemorrhage [10-12]. A recent meta-analysis indicated that LPL Ser447Ter polymorphism was associated with a significant reduction in the risk of stroke, especially atherosclerotic stroke subtype in both Caucasian and East-Asian [13].

The human LPL gene is situated on chromosome 8p22 and spans approximately 35 kb of DNA. It consists of 10 exons and 9 introns, which encode a mature protein of 448 amino acids. The LPL gene contains numerous variations, 80% of which occur in the coding regions [14]. A HindIII polymorphism, which results from a thymine (T) to guanine (G) substitutionis of particular interest because it is located in the intron 8, which is functionally unlike other common introns. As reported previously, the Hind III polymorphism may affect the transcription or translation of the LPL gene by interacting with regulatory elements in the 3' region [15]. But the relationship between LPL Hind III polymorphism and cerebral hemorrhage remains unclear.

In order to investigate the correlation between LPL Hind III polymorphism and cerebral hemorrhage, we detected the genotype of LPL Hind III polymorphism in both 300 cerebral hemor-

Table 1. Comparison of Clinical data between case and control groups

Parameters	n	Age (Years)	Gender (M/F)	BMI (Kg/m^2)	Diabetes, n (%)	Hypertension, n (%)	Smoking, n (%)	Alcohol drinking, n (%)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
CH group	300	60.2±10.1	160/140	22.2±3.4	89 (29.7)	92 (30.7)	102 (34.0)	49 (16.3)	1.88±0.9	4.9±1.1	1.6±0.3	2.9±10
Control group	300	60.3±11.0	163/137	22.6±3.4	41 (13.7)	43 (14.3)	42 (14.0)	38 (12.7)	1.31±0.5	4.6±1.1	1.5±0.4	2.1±0.8
P values		0.865	0.794	0.365	0.001	0.002	0.013	0.432	0.019	0.339	0.219	0.030

Figure 1. Genotyping result by PCR-RFLP. (1: TT genotype; 2: TG genotype; 3: GG genotype).
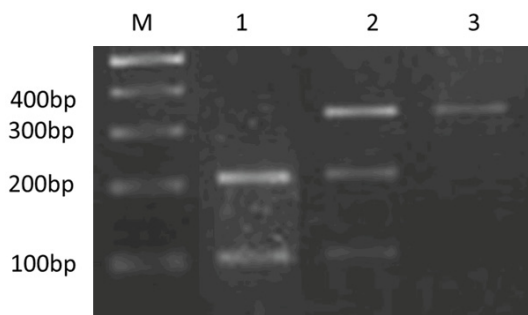

Figure 2. Sequencing results. (A: TT genotype; B: TG genotype; C: GG genotype).

rhage patients and 300 healthy people by PCR-RFLP method.

Materials and methods

Subjects

The cerebral hemorrhage patients were chose in Department of Neurosurgery, First Hospital of Hebei Medical University between Oct. 2012 and May 2013. 300 CH patients include160 males and 140 females with average age of (60.2±10.1) years. All CH patients were consistent with the diagnostic criteria of the Fourth National Cerebrovascular Disease Conference [16]. Head CT and (or) MRI was performed to confirm the patients' diagnosis. We excluded the patients with secondary and unexplained cerebral hemorrhage, peripheral vascular diseases or peripheral vascular thrombosis disease, artery disease, blood disease, tuberculosis, maliganttumorand severe liver and kidney dysfunction.

We also selected 300 healthy subjects including 163 males and 137 females from the same region as the CH patients. The average age of the control subjects was (60.3±11.0) years. The clinical data including previous medical history, smoking, drinking, etc. were collected by questionnaire. This research was based on the case-control study and got permission from Ethics Committee of First Hospital of Hebei Medical University.

Methods

DNA extraction

Genomic DNA was isolated with the E.Z.N.A. Blood DNA Midi Kit (Omega Bio-Tek, Norcross,
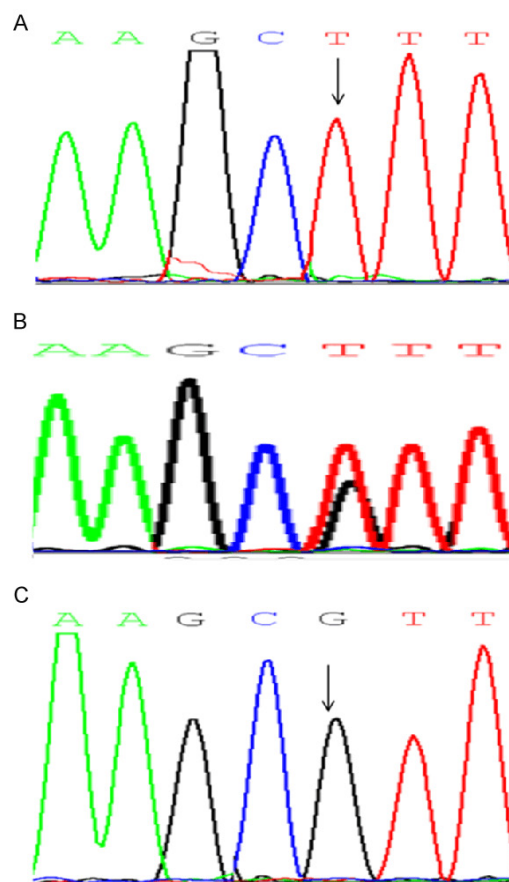
GA) from peripheral blood erythrocytes. The ultraviolet spectrophotometer was used to purify and quantify the DNA.

Genotyping

PCR-RFLP was used to amplify the Hind III polymorphism in intron 8 of the LPL gene. The primers used in this study refer to the previous study [17]. Sense primer: 5'-TTAGGGAACAAAC-CTCCG-3'; Antisense primer, 5'-CTGCCTTCAGC-TAGACATTG-3'. Each amplification reaction was performed with 22 μL of double-distilled water, 1 μL of each primer at 10 pmol/L, 25 μL of PCR mixture (0.05 U/μL Taq DNA polymerase reaction buffer, 4 mM of each dNTP), and 1 μL of template DNA, in a total volume of 50 μL. Amplification was performed in a Thermal Cycler 1000 instrument (Bio-Rad). An initial denaturation at 94°C for 3 minutes was followed by 40 cycles of denaturanon at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds. A fina-

Table 2. Hardy-Weinberg equilibrium analysis of Hind III SNPs in both CH and control subjects

Group		n	Hind III			χ^2	P value
			TT	TG	GG		
CH	Real	300	254	37	9	5.04	0.080
	prospective	300	247	50	3		
Control	Real	300	230	55	15	2.845	0.241
	prospective	300	221	67	9		

Table 3. Distribution of genotype and allele

Genotypes/ Allele	CH group, n (%)	Control group, n (%)	OR (95% CI)	P values
TT	254 (84.7)	230 (76.7)	1 (Ref.)	
TG	37 (12.3)	55 (18.3)	0.609 (0.387-0.959)	0.032
GG	9 (3.0)	15 (5.0)	0.543 (0.233-0.988)	0.041
T	545 (90.8)	515 (85.8)	1 (Ref.)	
G	55 (9.2)	85 (14.2)	0.611 (0.427-0.876)	0.007

lextension step was performed at 72°C for 5 minutes.

The PCR products were digested with Hind III restriction enzyme at 37°C for 16 hours. RFLP products were analyzed on 2% agarose gels that were stained with ethidium bromide.

Measurement of biochemical index

A 4 ml of fasting plasma was collected from each subject after at least 12 h of fasting. The plasma concentration of TC, TG, HDL-C, LDL-C, fasting blood glucose were detected according to the previous protocol [17].

Statistical analyses

SPSS 18.0 statistical software (SPSS, Chicago, IL, USA) was utilized to perform the statistical analyses. Hardy-Weinberg Equilibrium was tested by χ^2 test. Genotype and allele frequency were detected by χ^2-test. Measurement data were tested by t-test. The risk factors of cerebral hemorrhage were analyzed by logistic regression analysis. A P<0.05 was considered statistical significance.

Results

The clinical data comparison between two groups

There was no statistical difference inage, sex, body Mass Index, drinking history, TC, and HDL-C between the two group (all P>0.05). However, the diabetes mellitus, hypertension, smoking history, TG, and LDL-C, were different between the CH group and the control group (**Table 1**).

Genotyping results

As shown in **Figure 1**, the PCR amplification products has been demonstrated by agarose gel electrophoresis as a DNA fragment with 340 bp in length, then the fragment was digested by Hind III endonuclease and three kinds of genotype fragments was obtained: Wild homozygote genotype (TT) were 120 bp and 220 bp, mutant homozygote genotype (GG) were 340 bp, heterozygote genotype (TG) were 120 bp, 220 bp and 340 bp. To confirm the genotyping results, we also provided the sequencing results, as shown in **Figure 2**.

Distribution of genotype and allele between two groups

Genotype distributions were in line with Hardy-Weinberg Equilibrium both in the cerebral hemorrhage group and the control group (both P>0.05; **Table 2**). TT and TG genotype were 84.7% and 12.3% in the CH group, while ones were 76.7% and 18.3% in the control group, respectively. G allele frequency was significant lower in the CH group than that in the control group (OR=0.611; 95% CI: 0.427-0.876, P= 0.001). We also found both GG (OR=0.543, 95% CI: 0.233-0.988; P=0.041) and TG (OR=0.609, 95% CI: 0.387-0.959, P=0.032) genotype were frequent in the control group than that in the CH group (**Table 3**).

Comparison of blood lipid and other parameters between different genotype

TG level was much higher in TT genotype population than in TG+GG genotype population. It showed significant differences (P<0.05). The comparison on TC, HDL-C, and LDL-C showed no difference between the two groups (P>0.05, **Table 4**).

Table 4. Lipids profile comparison between each genotype

Parameters	TT genotype	GG+TG genotype	*P* values
TG (mmol/L)	1.94±0.92	1.44±0.63	0.020
TC (mmol/L)	4.61±0.64	4.42±0.91	0.514
HDL-C (mmol/L)	1.43±0.32	1.32±0.51	0.201
LDL-C (mmol/L)	2.73±1.02	2.61±0.93	0.310

Table 5. Logistic analysis results to adjust the confounders

Parameters	β	OR	95% CI	P
Smoking	1.781	3.241	1.102-7.209	0.004
Hypertension	1.198	2.101	1.671-6.211	0.002
Diabetes	1.319	3.778	1.420-8.265	0.019
Gallele	-0.676	0.601	0.231-0.876	0.001

Risk factors of cerebral hemorrhage analyzed by logistic regression

LPL Hind III G allele may be a protective factor after adjustment of age, hypertension and hyperglycemia by logistic multivariate regression analysis (OR=0.601, 95% CI: 0.231-0.876, P=0.001, **Table 5**).

Discussion

In the present study, we found G allele carriers have reduced risk for hemorrhagic stroke. In addition, we also found the plasma TG level, and LDL-C level increased significantly in the CH groupcompared to the control group. Further analysis based on the relationship between various genotypes and lipids metabolism showed that TG level of individuals who carrying Hind III (TT) was significantly higher than that of individuals carrying Hind III (TG) and Hind III (GG) gene.

The Hind III polymorphism is created by a thymine (T) to guanine (G) substitution, close to the 495th base position [15]. Previous studies have focused on the relationship between the Hind III polymorphism and levels of plasma TG, TC, HDL-C, and LDL-C [18-20]. The position of the Hind III polymorphism, within anintron, makes the mechanism by which it affects LPL activity unclear, but it is thought to prevent gene transcription. Some studies indicated that the Hind III polymorphism is in linkage disequilibrium with the S447X polymorphism, which is located in exon 9. However, despite the muta-

tions activating alternative splicing, which leads to the disease, the mutations may not be associated with the disease.

Some limitations in the present study should be mentioned. Firstly, the relatively small sample size may reduce the power. Secondly, we only investigated one SNP of the LPL gene. Therefore, the relation between other SNPs and CH remains unclear. Finally, we used a cross-sectional design in the present study, which is difficult to clarify the causality between LPL genetic polymorphism and CH.

Nevertheless, our study reached the interesting conclusion that the G allele of the Hind III polymorphism has an impact on levels of TG in the plasma and appears to have a protective effect against CH. However, our results should be confirmed by a large-scale case-control study or cohort study in the future.

Disclosure of conflict of interest

None.

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