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Original Article

Lipoprotein lipase gene Hind III polymorphism was associated with hemorrhagic stroke

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Abstract: Objective: To investigate the relevance between lipoprotein lipase (LPL) Hind III gene polymorphism and cerebral hemorrhage. Methods: A case-control study was performed utilizing PCR-RFLP method and sequencing of amplified products to detect LPL Hind III gene polymorphism in 350 cases of hemorrhagic stroke (HS group) and 350 healthy subjects (control group). Blood lipids and glucose levels were also recorded for each attendant. Results: In HS group, T and G allele frequencies were 90.8% and 9.2%, respectively; while those in the control group were 82.3% and 17.7%. In HS group, detection rate of the G allele frequency and GG genotype were significantly lower than those in the control group. In addition, TG, LDL-C, fasting blood glucose , systolic blood pressure , diastolic blood pressure were significantly higher in HS group ($P<0.05$, $P<0.01$). Compared with TG+GG genotype, TT genotype population show significantly higher triglycerides concentration ($P<0.05$). With adjustment for hypertension, high blood sugar, and age -related factors, multivariate logistic regression analysis showed that LPL Hind III G allele could be a protective factor (OR = 0.392, 95% CI: 0.191~0.805, P = 0.011). Conclusion: LPL Hind III gene polymorphism was relevant to hemorrhagic stroke. LPL Hind III G mutant allele could be a protective factor in the pathogenesis of cerebral hemorrhage.

Keywords: Cerebral hemorrhage, lipoprotein lipase, polymerase chain reaction, gene frequencies, genotype, risk factors

Introduction

Previous studies have shown that lipoprotein lipase (LPL) anomalies were associated with hyperlipidemia, atherosclerosis coronary heart disease, stroke, diabetes, chronic kidney disease and cancer [1-4]. Hemorrhagic stroke is the more common form of stroke type. Some previous studies have indicated that the genetic polymorphisms in APOE, ACE and AT1R genes were associated with hemorrhagic stroke [5-7].

Several studies suggested that Hind III polymorphism in LPL gene was related to serum lipid levels but the conclusion is still controversial [8-11]. Munshi et al [8] found that Hind III polymorphism TT genotype carriers have increased serum triglyceride and glucose levels compared with the carriers with GG and TG genotype in South India. He et al [9] also found that patients carrying Hind III TT genotype have increased TC levels and reduced HDL-C levels. However, Xu et al [10] and Guan et al. [11] found that T allele was not associated with plasma TG, TC, HDL-C, and LDL-C levels. In addition, previous study suggested LPL Hind III polymorphism was related to cerebral infarction [10, 12-14]. But the relationship between LPL Hind III genetic polymorphism and hemorrhagic stroke is unclear.

The present study aims to explore the relation between LPL Hind III gene polymorphism and hemorrhagic stroke in Chinese Han population.

Subjects and methods

Subjects

We performed a case-control study involving 700 subjects (350 cases and 350 controls). All the hemorrhage stroke patients were selected from Qilu Hospital, Shandong University

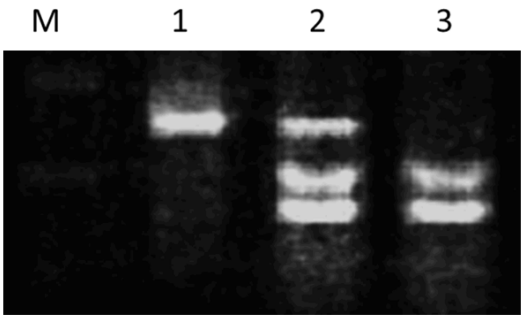

Figure 1. Genotyping results of PCR-RFLP; M: DNA marker; 1: GG genotype; 2: TG genotype; 3: TT genotype.

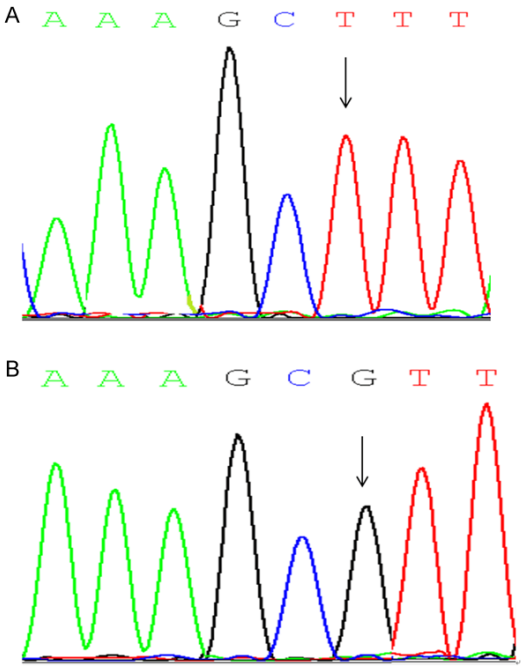

Figure 2. Sequencing results of Lipoprotein lipase gene Hind III polymorphism. A: T allele; B: G allele. Arrow: Mutation locus.

between October 2011 and January 2014. 350 hemorrhage stroke including 198 cases of male and 152 females, with an average age (62.3±12.2) years. All the patients were diagnosed according to the disease classification and diagnostic criteria for stroke in 1998 [15]. In addition, all the patients were confirmed by CT and (or) MRI.

Exclusion criteria: we excluded patients with secondary and unexplained cerebral hemorrhage, with peripheral vascular disease or peripheral vascular occlusive diseases, aortic diseases, blood diseases, tuberculosis, cancer, serious liver and kidney dysfunction.

We selected 350 sex- and age-matched healthy subjects as the controls (188 cases of male and 162 cases of females), with an average age (62.4±11.9) years. We collected the clinical data including previous disease history, smoking, and alcohol consumption etc.

This study was approved by the ethics committee of Qilu Hospital, Shandong Medical University.

Methods

DNA extraction: We collected 2 ml of fasting venous blood with anticoagulation of EDTA in each participant. We extracted genomic DNA Using BLOOD DNA MIDI KIT (OMEGA, Chicago, IL, USA) according to the instruction.

Genotyping: PCR primers used for genotyping were asfollows: a Sense primer sequence: 5'-TTAGGGAACAAACCTCCG-3', Antisense primer sequence: 5'-CTGCCTTCAGCTAGACATTG-3'.

PCR reaction in a final volume was 50 uL, including 1.5 ul oftemplate DNA, 2 uL of primer, 25 uL of PCRMix, 8 uL of deionized water. Amplification conditions: denaturation at 94°C for 3 min, following 30 cycles: 94°C for 30 s, annealing 57°C for 30 s, extension 72°C for 50 s; final extension at 72°C for 5 min. PCR products was digested with Hind III restriction enzyme for 16 h at 37°C water bath. The reaction system was as follows: PCR amplification product 10 uL, Hind III restriction enzyme 1.5 U, 10 × Buffer 2 uL, deionized water 18 uL.

Biochemical detection: 4 ml of fasting peripheral blood were collected. The serum TC, TG, HDL-C, LDL-C, fasting blood glucose, apolipoprotein A (apoA) and apoB were detected according to previous literature.

Statistical analysis

SPSS 13.0 statistical software was utilized to analyze the data. Hardy-Weinberg equilibrium was tested by X^2 test, measurement data were compared using *t* test. Logistic regression analysis was used to adjusted confounders. A $P<0.05$ was considered statistically significant.

Table 1. The clinical characteristics comparison between case and control group

Indices	HS group	Control group	*P* values
Age (Years)	62.3±12.2	62.4±11.9	0.771
Gender (M/F)	198/152	188/162	0.753
BMI (Kg/m^2)	23.5±3.4	23.6±3.8	0.218
Diabetes, n (%)	101 (28.9)	46 (13.1)	0.014
Smoking, n (%)	122 (34.9)	68 (19.4)	0.022
Alcohol drinking, n (%)	63 (18.0)	61 (17.4)	0.432
TG (mmol/L)	1.75±0.8	1.41±0.7	0.010
TC (mmol/L)	4.6±0.9	4.5±1.0	0.339
HDL-C (mmol/L)	1.4±0.4	1.3±0.3	0.219
LDL-C (mmol/L)	3.4±1.1	2.4±0.9	0.015
Blood glucose (mmol/L)	6.2±2.3	5.1±1.5	0.003
Apo A (mmol/L)	1.5±0.4	1.4±0.6	0.122
Apo B (mmol/L)	0.9±0.3	1.0±0.4	0.331
SBP (mmHg)	158.1±25.5	125.7±11.3	<0.001
DBP (mmHg)	92.4±14.4	82.3±10.1	<0.001

Table 2. Distribution of genotype and allele

Genotypes/Allele	HS group, n (%)	Control group, n (%)	*P* values
TT	301 (86.0)	274 (78.3)	0.028
TG	43 (12.3)	66 (18.9)	
GG	6 (1.7)	10 (2.8)	
T	640 (91.4)	614 (87.7)	0.001
G	60 (8.6)	86 (12.3)	

Results

Genotyping results

As shown in **Figure 1**, there were three genotypes (TT, TG, and GG) in both patients and control subjects. We also performed direct sequence to confirm the genotyping results (**Figure 2**).

Clinical characteristics of the participants

There were not statistically significant in age, gender, body mass index, alcohol consumption history, TC, HDL-C, apoA and apoB between the case and control group (all P>0.05). However, there were significant difference in diabetes, smoking history, TG, LDL-C, fasting blood glucose, systolic blood pressure, and diastolic blood pressure was significantly difference between the two groups (all P<0.05 or P<0.01, **Table 1**).

Genotype and allele distribution between the two groups

The genotype distribution both in hemorrhagic stroke and control groups were consistent with Hardy-Weinberg equilibrium (both P>0.05). The GG genotype frequency was 0.8% and 2.7% in hemorrhagic stroke and control groups, respectively. The TG and TT genotype frequency were 82.5% and 16.7%, respectively, in the hemorrhagic stroke group; while ones were 67.3% and 29.9% in the control group. The difference was significant (P<0.05). The allele T and G frequency was 90.8% and 9.2% in hemorrhagic stroke group, but 82.3% and 17.7% in the control group, the difference was statistically significant (P<0.05, **Table 2**).

Different genotypes compared with other lipid parameters

Compared with carrier with G allele, TT genotype carriers have increased level of serum TG concentration (P<0.05). We did not found statistically significant in TC, HDL-C, LDL-C, apoA, apoB, fasting glucose, systolic blood pressure, and diastolic blood pressure between each genotype (**Table 3**).

Logistic regression analysis

Multivariate logistic regression analysis showed that, by adjusting for age, high blood pressure, high blood sugar and other risk factors, LPL Hind III G allele may be a protective factor for hemorrhagic stroke (OR = 0.953, 95% CI: 0.191~0.805, P = 0.011, **Table 4**).

Discussion

The present study showed that LPL Hind III polymorphism was associated with both hemorrhagic stroke risk and lipids levels in Chinese Han population. Our results were in line with the previous report that suggested the G allele may have a beneficial effect on the level of serum TG and hemorrhagic stroke.

Currently, the correlation between Hind III genotype and hemorrhagic stroke was unclear. Several previous study reported controversial conclusion. In the present study, we find G allele frequency in the hemorrhagic stroke was lower than that in the control groups. Further

Table 3. Clinical characteristics comparison between each genotype

Indices	TT genotype	GG+TG genotype	*P* values
TG (mmol/L)	1.8±0.8	1.3±0.6	0.014
TC (mmol/L)	4.7±0.8	4.6±0.9	0.577
HDL-C (mmol/L)	1.4±0.3	1.4±0.5	0.41
LDL-C (mmol/L)	3.5±1.0	3.4±0.9	0.321
Apo A (mmol/L)	1.5±0.6	1.4±0.9	0.322
Apo B (mmol/L)	0.9±0.4	1.0±0.3	0.232
Blood glucose (mmol/L)	6.1±2.1	6.2±1.8	0.798
SBP (mmHg)	159.2±23.2	155.3±14.2	0.119
DBP (mmHg)	92.5±15.1	92.3±13.2	0.71

Table 4. Multivariate Logistic analysis

Indices	β	OR	95% CI	*P*
Smoking	1.981	4.231	1.342-8.221	0.012
SBP	1.230	3.121	1.221-5.212	<0.001
DBP	1.012	2.121	1.120-5.101	<0.001
Blood glucose	1.303	2.104	1.139-6.219	<0.029
GG+TG genotype	-0.932	0.432	0.190-0.902	0.013

logistic regression analysis showed that G allele may be a protective factor for hemorrhagic stroke. Hind III polymorphism is located on intron 8, the following mechanisms may affect the function of LPL: 1) There may be another functional variant in high linkage disequilibrium with Hind III in the region of LPL gene; 2) The Hind III variation may affect the splicing site, which affects the LPL gene function. Previous studies have shown that another variation Ser 447 stop, being located on the exon 9 of LPL gene, was associated with serum TG and HDL-C levels [16]. Ser 447 mutation may be an independent risk factor for hyperlipidemia [17], coronary heart disease [18] and cerebrovascular disease [13]. Furthermore, this locus is very close to the locus Hind III location, which indicated that there may be a linkage disequilibrium between the two loci. In addition, the Pvu II locus in intron 6 of LPL gene also was significantly correlated to lipid levels [19]. It is still unclear if there is a linkage disequilibrium between the two loci.

Current studies indicated that there are significant difference in race and region of LPL gene. Therefore, the relation between Hind III locus and hemorrhagic stroke are worthy of exploring in different race and region in future study.

In conclusion, the present study indicated that LPL Hind III genetic polymorphism was associated with hemorrhagic stroke. G allele may be a protective factor of hemorrhagic stroke in Chinese population.

Disclosure of conflict of interest

None.

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