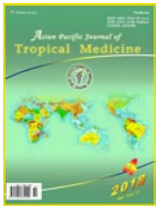

IF: 0.925

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.231473

Tag single nucleotide polymorphism rs1532624 located in cholesteryl ester transfer protein gene is associated with atherosclerosis cerebral ischemia

Lin Huang[1#], Dan-Xin Wang[2#], Li-Min Zhou[3#], Tao Wang[2], Hai-Ying Zhang[3✉], Yun-Xia Zhang[3✉], Yin-Dong Zhang[1✉]

[1]*Institute of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China*
[2]*Department of Nursery, The Affiliated Hospital of Hainan Medical College, Haikou 571199, China*
[3]*Public Research Laboratory of Hainan Medical University, Haikou 571199, China*

ARTICLE INFO

Article history:
Received 1 January 2018
Received in revised form 18 February 2018
Accepted 12 March 2018
Available online 2 April 2018

Keywords:
Cholesteryl ester transfer protein gene
Atherosclerotic cerebral infarction
Gene polymorphism

ABSTRACT

Objective: To investigate the relationship between polymorphisms of rs1532624 and rs289741 loci in cholesteryl ester transfer protein (*CETP*) genes and atherosclerotic cerebral infarction (ACI). **Methods:** The *CETP* gene rs1532624 and rs289741 in 95 patients with ACI and 177 healthy subjects were genotyped by MassARRAY mass spectrometry. Each locus genotype and allele frequency distributions were compared. **Results:** The difference of allele frequency distribution between the rs1532624 (χ^2=1.723, P=0.189) and rs289741 (χ^2=2.466, P=0.116) were not statistically significant. The frequency distribution of rs1532624 genotype between the cerebral infarction group and healthy control group was statistically significant (χ^2=7.096, P=0.029), while rs289741 genotype frequency distribution between the two groups was not statistically significant (χ^2=2.906, P=0.234). **Conclusion:** ACI have a positive correlation with rs1532624 polymorphism, and AA genotype may be susceptible factors of ACI.

1. Introduction

Cholesteryl ester transfer protein (CETP) is a single-chain, highly hydrophobic glycoprotein with a molecular weight of 74 kDa[1]. CETP is a lipid carrier between lipoproteins, playing an important role in cholesterol retrograde transport system and thus promoting the exchange of neutral fat and phospholipids in plasma lipoproteins[2-4]. Studies have shown that genetic polymorphisms can affect CETP activity, thereby affecting lipid metabolism and leading to the formation of atherosclerosis.

Atherosclerosis is an important cause of cerebral infarction. As one of the susceptible genes[5], *CETP* gene plays a decisive role in lipid transport activity. At present, more and more polymorphic sites have been found[6]. In this study, two polymorphic loci of rs1532624 and rs289741 in *CETP* gene were selected to analyze the association between gene polymorphism and atherosclerotic cerebral infarction (ACI).

#These authors contributed equally to this work.
First author: Lin Huang, Institute of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China.
E-mail: 290584459@qq.com
✉Corresponding author: Dr. Hai-Ying Zhang, Public Research Laboratory of Hainan Medical University, Haikou 571199, China.
Tel/Fax: +86-898-66892235
E-mail: 842140511@qq.com
Dr. Yun-Xia Zhang, Public Research Laboratory of Hainan Medical University, Haikou 571199, China.
E-mail: zhangyunxia1977@qq.com
Tel/Fax: +86-898-66892235
Dr. Yin-Dong Zhang, Institute of Tropical Agriculture and Forestry, Hainan university, Haikou 570228, China.
Tel/Fax: +86-898-66272079
E-mail: 23300558@163.com
Fund project: The study was supported by grants from Natural Science Foundation of China (31501018, 31760310, and 81660224), and the Social Development Project of Hainan Province (SF201401).

How to cite this article: Huang L, Wang DX, Zhou LM, Wang T, Zhang HY, Zhang YX, Zhang YD. Tag single nucleotide polymorphism rs1532624 located in cholesteryl ester transfer protein gene is associated with atherosclerosis cerebral ischemia. Asian Pac J Trop Med 2018; 11(4): 309-312.

2. Materials and methods

2.1. Research objects

Cerebral infarction group: 95 cases of cerebral infarction were selected from Cadre Sanatorium of Hainan Province, including 51 males and 44 females, with average age (67.8±11.2). All patients met the criteria of International Classification of Diseases 10th Edition (ICD10). Patients with persistent, sudden onset of focal neurological deficits above 24 h were diagnosed by head CT and/or head MRI and vascular imaging.

Healthy control group: 177 healthy subjects with the same physical examination center in Cadre sanatorium of Hainan Province, including 116 males and 61 females, with an average age of (68.9±10.4) years. All healthy people had no coronary heart disease or past cerebral infarction and no family history of cerebrovascular disease. All subjects were from Hainan Province and had no blood relationship with each other. This study was approved by the Hospital Ethics Committee, and all participants signed an informed consent form.

2.2. Blood collection and DNA extraction

Fasting venous blood samples were drawn into 2 mL EDTA anticoagulation tube. DNA extraction was done by Genomic DNA extraction kit, and the products was stored at -20 ℃.

2.3. Genetic polymorphism detection

DNA sequencing and primer designed were accomplished by Shenzhen Huada Gene Technology Services Ltd., using ADS2.0 software design primers (Pre-PCR primer and Extension primer, Table 1) and MassARRAY mass spectrometry single nucleotide polymorphism (SNP) typing method.

Pre-PCR reaction system: total system is 5.0 μL, containing template DNA 1.0 μL, 10× PCR Buffer 0.5 μL, 25 mmol/L $MgCl_2$ 0.4 μL, 25 mmol/L dNTP mix 0.1 μL, Primer mix 1.0 μL, 0.5 U HotstarTaq 0.1 μL, and H_2O 1.9 μL. PCR reaction program: denaturation at 94 ℃ for 2 min, denaturation at 94 ℃ for 20 s, annealing at 56 ℃ for 30 s, extension at 72 ℃ for 60 s, 45 cycles and extension at 72 ℃ for 5 min.

SAP digestion reaction system and procedures: SAP buffer 0.17 μL, SAP enzyme 0.30 μL, H_2O 1.53 μL, 37 ℃ water bath for 40 min, 85 ℃ inactivated 5 min.

EP reaction system: H_2O 0.62 μL, iPLEX buffer 0.20 μL, iPLEX Termination mix 0.20 μL, iPLEX Extend Primer mix 0.94 μL, and iPLEX enzyme 0.04 μL. EP reaction program: 94 cycles 2 min, 40 cycles × 52 ℃ 5 s, 80 ℃ 5 s, 72 ℃ 3 min. Then the extension product was purified by resin, spotted on the machine, and genotyped by mass spectrometer.

2.4. Statistical analysis

SPSS16.0 software was used for statistical analysis. Deference of genotypes and allele frequencies between groups was tested by χ^2 test with a=0.05.

3. Results

3.1. Analysis of rs1532624 and rs289741 polymorphism

The results of MassARRAY mass spectrometry showed that the genotypes of rs1532624 in 95 patients with cerebral infarction and 177 healthy controls were CC, CA, and AA. CC and AA showed a single peak at Mass 4 824.2 and Mass 4 864.1, respectively. However, the CA genotypes had bimodal peaks at Mass 4 824.2 and Mass 4 864.1 (Figure 1).

The genotypes of rs289741 were AA, AG, and GG. AA and GG showed a single peak at Mass 4 809.2 and Mass 4 825.2, respectively, but the AG genotype showed double peaks at Mass 4 809.2 and Mass 4 825.2 (Figure 2).

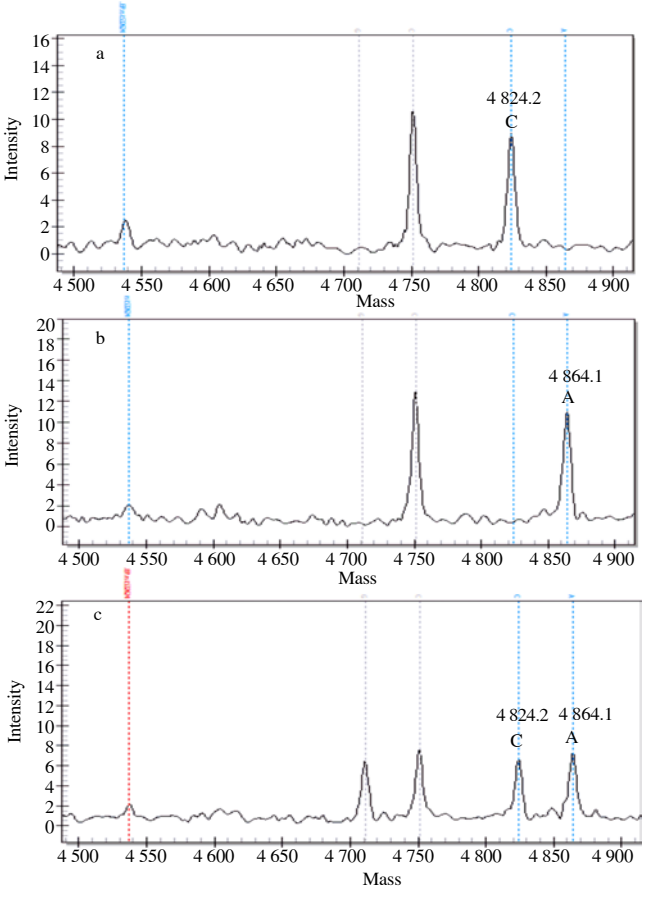

Figure 1. Detection for genotypes of rs1532624.

The CC and AA genotypes show a single peak at Mass 4 824.2 (a) and Mass 4 864.1 (b) respectively, while the CA genotype shows bimodal peaks at Mass 4 824.2 and Mass 4 864.1 (c).

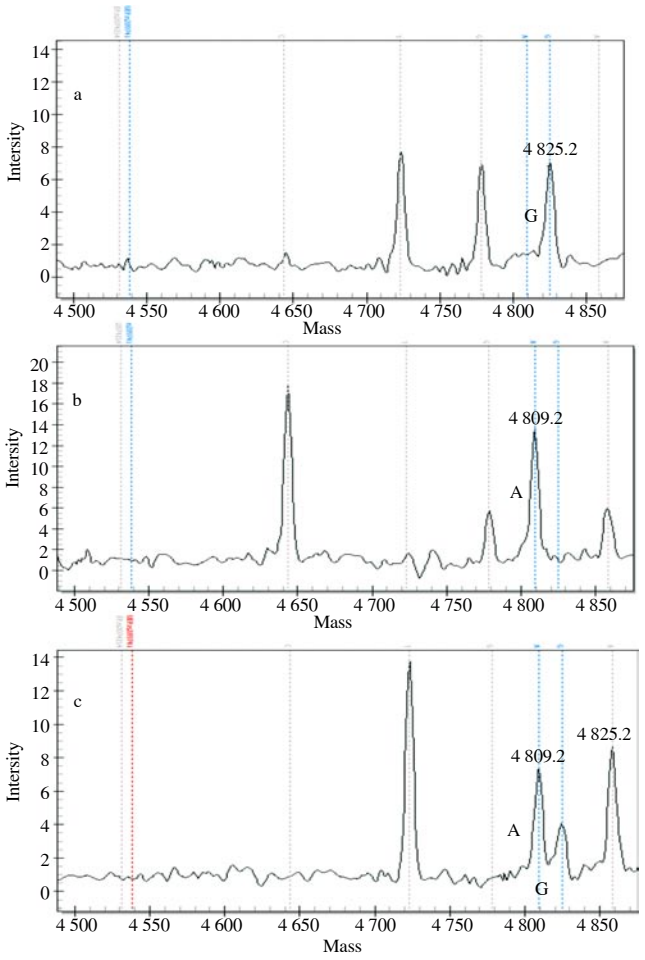

Figure 2. Detection for genotypes of rs289741.

The GG and AA genotypes show a single peak at Mass 4 825.2 (a) and Mass 4 809.2 (b), respectively, while the AG genotype shows double peaks at Mass 4 809.2 and Mass 4 825.2 (c).

3.2. Correlation analysis of rs1532624 and rs289741 locus polymorphism and ACI

There was no significant difference in allele frequency distribution between rs1532624 (χ^2=1.723, *P*=0.189) and rs289741 (χ^2=2.466, *P*=0.116) in the cerebral infarction group and healthy control group. The frequency distribution of rs1532624 genotype between the cerebral infarction group and healthy control group was statistically significant (χ^2=7.096, *P*=0.029), while rs289741 genotype frequency distribution between the two groups was not statistically significant (χ^2=2.906, *P*=0.234) (Table 2).

4. Discussion

Atherosclerosis refers to the chronic inflammatory process of lipid deposition, extracellular matrix proliferation, and cell infiltration in cardiovascular and cerebrovascular diseases. It can cause stenosis and plaque loss in the arterial lumen, leading to ischemia and hypoxia necrosis of brain tissue[7]. ACI is a complex disease that interacts with many environmental and genetic factors. Atherosclerosis is the most basic pathological feature. With the development of molecular biology and genetics, the study of the correlation between gene polymorphism and disease has become a hot spot. In this study, *CETP* gene, which is closely related to lipid transport activity, was selected to investigate the association between rs1532624 and rs289741 polymorphisms and ACI in Hainan population.

The human *CETP* gene is near the lecithin cholesterol acyltransferase gene and consists of 16 exons and 15 introns at 16q21, about 25 kbp in length. Mature CETP protein consists of 476 amino acid residues, of which 45% hydrophobic amino acids, is a hydrophobic glycoprotein[8,9]. The process of reverse cholesterol transport refers to the process by which plasma high density lipoprotein (HDL) transports the excess cholesterol in the body to the liver for further metabolism. Cholesterol reverse transport process can reduce plasma cholesterol levels and thus is considered to have anti-atherosclerotic function, and CETP plays a key role in the reverse cholesterol transport process. CETP can also change the level of HDL-cholesterol and HDL particle size by mediating cholesterol from HDL to apolipoprotein-containing very low (VLDL) and low density lipoprotein (LDL) conversion[10-14]. *CETP* gene mutations can affect its activity, thereby changing the concentration of plasma

Table 1

Pre-PCR prime and extension primer.

SNPs	Pre-PCR prime	Extension primer
rs1532624	F: 5′-ACG TTG GAT GCA CCC ATT TGT CCT GAG TTC-3′	R: 5′-CCA CAC AGC TTG TGA-3′
	R: 5′-ACG TTG GAT GAC TTT GGC AAA TCT CTG CCC-3′	
rs289741	F: 5′-ACG TTG GAT GTC TAC CAG CTT GGC TCC CTC-3′	F: 5′-GAG TCA GCC CAG CTC-3′
	R: 5′-ACG TTG GAT GCA TCT GCA GCA GCA GGA AG-3′	

SNPs: single nucleotide polymorphisms.

Table 2

Genotype and allele frequency distribution comparison of rs1532624 and rs289741 loci [*n*(%)].

Group	rs1532624					rs289741				
	CC	CA	AA	C	A	AA	AG	GG	A	G
Healthy control group	89(50.3)	83(46.9)	5(2.8)	261(73.7)	93(26.3)	84(47.5)	79(44.6)	14(7.9)	247(69.8)	107(30.2)
Cerebral infarction group	45(47.4)	40(42.1)	10(10.5)	130(68.4)	60(31.6)	35(36.9)	50(52.6)	10(10.5)	120(63.2)	70(36.8)
χ^2		7.096		1.723			2.906		2.466	
P value		0.029		0.189			0.234		0.116	

HDL and particle size and promoting or resisting the occurrence and development of atherosclerosis[13].

For rs1532624 polymorphic locus, the results showed that there was no significant difference between the C allele frequency of cerebral infarction group and the healthy control group (68.4% *vs*. 73.7%, χ^2=1.723, *P*=0.189). The frequency of AA genotype in the cerebral infarction group was 10.5% higher than AA genotype in healthy control group (2.8%), with statistical significance (χ^2=7.096, *P*=0.029). This suggests that there may be a correlation between *CETP* rs1532624 polymorphism and ACI, and AA genotypes may be risk factors of ACI and promote the occurrence and development of ACI.

However, the rs289741 locus had no significant difference in genotype frequency and allele frequency between the cerebral infarction group and healthy control group (χ^2=2.906, *P*=0.234; χ^2=2.466, *P*=0.116 respectively). Therefore, we cannot draw the conclusion that the rs289741 polymorphism was associated with ACI and it may be caused by small sample bias.

In summary, the study obtained some results, but the sample size collected in this study was small. A single factor analysis is not enough to represent all patients. Thus, in order to explore the exact relationship between *CETP* gene polymorphism and ACI, we need to further expand the sample size and systematically study the larger genetic background population.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Acknowledgments

The study was supported by grants from Natural Science Foundation of China (31501018, 31760310, and 81660224), and the Social Development Project of Hainan Province (SF201401).

References

[1] Bruce C, Chouinard RA, Tall AR. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu Rev Nutr* 1998; **18**: 297-330.

[2] Sandhofer A, Tatarczyk T, Laimer M, Ritsch A, Kaser S, Paulweber B, et al. The Taq1B-variant in the cholesteryl ester-transfer protein gene and the risk of metabolic syndrome. *Obesity (Silver Spring)* 2008; **16**(4): 919-922..

[3] Ritter MC, Bagdade JD. Contribution of glycaemic control, endogenous lipoproteins and cholesteryl ester transfer protein to accelerated cholesteryl ester transfer in IDDM. *Eur J Clin Invest* 1994; **24**: 607-614.

[4] Hesler CB, Tall AR, Swenson TL, Weech PK, Marcel YL, Milne RW. Monoclonal antibodies to the Mr 74,000 cholesteryl ester transfer protein neutralize all of the cholesteryl ester and triglyceride transfer activities in human plasma. *J Biol Chem* 1988; **263**(11): 5020-5023.

[5] Pillois X, Phuong Do Thi N, Reynaud A, Benchimol D, Lagrost L, Bonnet J. TaqIB polymorphism in cholesterol ester transfer protein (*CETP*) gene predicts future cardiovascular death in patients experiencing an acute coronary syndrome. *Clin Chem Lab Med* 2009; **47**(9): 1039-1046.

[6] Zhao KR, Ren NY, Zhang WF, jin B, Qian JJ. Correlative study on intima-media thicknessof carotid artery in cerebral infarction by colour Doppler ultrasound. *Clin Med Engin* 2013; **20**: 129-131.

[7] Agellon LB, Quinet EM, Gillette TG, Drayna DT, Brown ML, Tall AR. Organization of the human cholesteryl ester transfer protein gene. *Biochemistry* 1990; **29**(6): 1372-1376

[8] Stevenson SC, Wang S, Deng L, Tall AR. Human plasma cholesteryl ester transfer protein consists of a mixture of two forms reflecting variable glycosylation at asparagine 341. *Biochemistry* 1993; **32**(19): 5121-5126.

[9] Grundy SM, Pasternak R, Greenland P, Smith S Jr, Fuster V. AHA/ACC scientific statement: Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 1999; **34**(4): 1348-1359.

[10]Charles MA, Kane JP. New molecular insights into *CETP* structure and function: A review. *J Lipid Res* 2012; **53**(8): 1451.

[11]Barter PJ, Kastelein JJ. Targeting cholesteryle ester transferprotein for the prevention and management of cardiovascular disease. *Am Coll Cardol* 2006; **47**(3): 492-499.

[12]McCaskie PA, Beilby JP, Chapman CM, Hung J, McQuillan BM, Thompson PL, et al.Cholesteryl ester transfer protein gene haplotypes, plasma high-density lipoprotein levels and the risk of coronary heart disease. *Hum Genet* 2007; **121**(3-4): 401-411.

[13]Zhou HX, Gao LH, Meng LL, Zhang YX, Wei ZF, Si DW. Preventive and therapeutic effect of simvastatin on secondary inflammatory damage of rats with cerebral hemorrhage. *Asian Pac J Trop Med* 2017; **10**(2): 146-150.

[14]Fang H, Zhang JC, Yang M, Li HF, Zhang JP, Zhang FX, et al. Perfusion of gastrodin in abdominal aorta for alleviating spinal cord ischemia reperfusion injury. *Asian Pac J Trop Med* 2016; **9**(7): 678-683.