Relationship between *ABCA1* gene polymorphism and lacunar infarction combined with arteriosclerosis in patients

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Abstract. Adenosine triphosphate-binding cassette transporter A1 *(ABCA1)* gene polymorphism in lacunar infarction (LI) combined with arteriosclerosis was investigated. A total of 112 LI patients complicated with arteriosclerosis treated in Ningbo First Hospital from March 2015 to September 2016 were enrolled as observation group. At the same time, 342 healthy subjects were selected from physical examination center to serve as the control group. The *ABCA1* gene polymorphism was detected via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the susceptibility of *ABCA1* gene to LI complicated with atherosclerosis was studied. There were no significant differences in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and lipoprotein (a) [Lp(a)] between the two groups (P>0.05). Levels of triglyceride (TG) and apolipoprotein B (ApoB) in observation group were significantly higher than those in control group, but levels of ApoA-I and high-density lipoprotein cholesterol (HDL-C) were significantly lower in observation group than those in control group (P<0.05). There were no significant differences in the RR, RK and KK frequencies and allele frequency of ABCA1 R219K genotype between the two groups (P>0.05). Moreover, levels of HDL-C increased in the RR, RK and KK genotypes, but were not statistically significant (P>0.05). Levels of TG, TC, LDL-C, ApoA-I, ApoB and Lp(a) showed no significant differences among different genotypes of *ABCA1* R219K (P>0.05). Results indicated that *ABCA1* R219K polymorphism has no correlation with LI complicated with arteriosclerosis.

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Introduction

As a type of cerebrovascular disease, lacunar infarction (LI) seriously threatens the health and life of the middle aged and elderly people. LI accounts for approximately 30% of cerebral infarctions, and incidence rate of LI shows an increasing trend year by year. Adenosine triphosphate-binding cassette transporter A1 (ABCA1) is an integral membrane protein with ATP as the energy resource. ABCA1 can promote the release of free phospholipids and free cholesterol from cells. The released phospholipids and cholesterol will bind to apolipoprotein A1 (ApoA-I) on the cell surface to form high-density lipoprotein cholesterol (HDL-C), thus being involved in the reverse transport of cholesterol in the human body and playing an anti-atherosclerosis role (1,2). Related data have shown (3,4) that *ABCA1* gene mutation can induce HDL-C deficiency accompanied by atherosclerosis. *ABCA1* gene single nucleotide polymorphism (SNP) is closely related to the development of atherosclerosis and plasma lipid level (5), and *ABCA1* R219K is the common SNP (6-8). At present, the roles of *ABCA1* R219K in LI complicated with atherosclerosis still have not been well studied.

Patients and methods

General materials. A total of 112 LI patients complicated with arteriosclerosis treated in Ningbo First Hospital from March 2015 to September 2016 were enrolled as observation group. Inclusion criteria: i) patients who met the diagnostic criteria of LI confirmed via magnetic resonance imaging (MRI) or computed tomography (CT); ii) patients who were complicated with arteriosclerosis iii) patients who signed the informed consent. Exclusion criteria: i) patients with severe dysfunctions in liver, kidney or other organs; ii) patients with cancer. At the same time, 342 healthy volunteers were selected from physical examination center to serve as control group. There were no significant differences in the general information between the two groups (P>0.05) (Table I).

Diagnostic criteria for LI: i) Acute lacunar infarction can be seen as a circle, ellipse or fissure with clear boundary on diffusion-weighted imaging (DWI). T1MI showed low intensity signal. TT2WI and FLAIR sequence images showed high intensity signal. ii) In chronic lacunar infarction, the signal

Table I. General data of the patients and the controls.

Items	Observation group (n=112)	Control group (n=342)	t/χ^2 value	P-value
Sex (male/female)	59/53	174/168	0.049	0.824
Age (years)	20-59	20-60		
Average age (years)	68.85±7.89	68.37±7.48	0.581	0.562
Body mass index (kg/m^2)	23.55±2.37	23.42±2.53	0.479	0.632
Educational level				
Junior high school and below	15 (13.39)	43 (12.57)	0.102	0.951
Senior high school and technical secondary school	59 (52.68)	178 (52.05)		
Junior college and above	38 (33.93)	121 (35.38)		

Table II. Primers used in PCR reactions.

Genes	Sequences
ABCA1	F: 5'-ACCGAAGTAAGGAGTTGCTCATA-3'
	R: 5'-GTGATATGGCATCGTTGCATTT-3'
β-actin	F: 5'-ACTGGCATTGTGATGGACTC-3'
	R: 5'-AGGAAGGAAGGCTGGAAGAG-3'

F, forward; R, reverse.

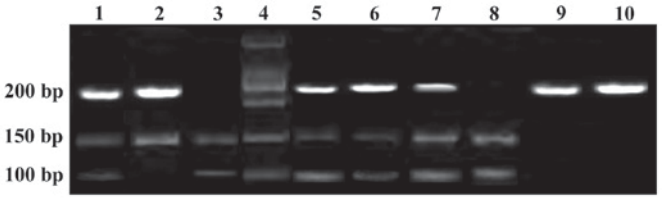

Figure 1. Separation of digested ABCA1 R219K PCR product by 8% polyacrylamide gel electrophoresis; lanes 1, 2, 5, 6 and 7, RK genotype; 3 and 8, KK genotype; 9 and 10, RR genotype; 4, pUC19 DNA/MSPI Marker.

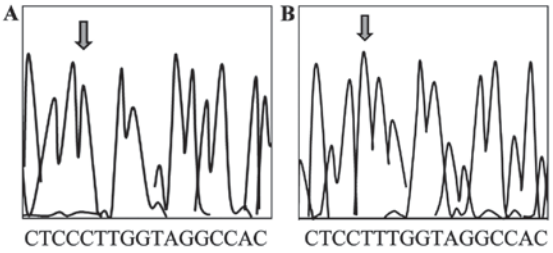

Figure 2. PCR product sequencing map. Base C (indicated by upper arrow) in RR genotype and base T (indicated by lower arrow) in KK genotype of ABCA1 PCR amplification product.

intensity was low on both T1MI and FLAIR images, and the diameter was <20 mm. iii) There was no cognitive impairment before onset of lacunar infarction.

Diagnostic criteria for arteriosclerosis: Carotid artery medial thickness >1.00 mm.

Biochemical detection. Venous blood (10 ml, fasting for 8 h) was collected from each participant, followed by centrifugation at 2,500 x g for 5 min to collect serum. Serum samples were stored at -70˚C before use. Levels of serum triglyceride (TG), total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), ApoA-I were detected using a full-automatic biochemical analyzer (model BS-800; Mindray Medical International Ltd., Shenzhen, China). The Lp(a) and ApoB was detected by ELISA (enzyme linked immunosorbent assay).

Genomic DNA extraction and polymerase chain reaction (PCR) amplification. Venous blood (3 ml, fasting for 8 h) was collected from each participant of two groups, and placed in ethylene diamine tetraacetic acid (EDTA) anticoagulant tube to extract the genomic DNA using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the instructions. The amplification was performed with a 25 μl reaction system using the PCR amplification instrument (Shanghai Huanxi Medical Co., Ltd., Shanghai, China). Primers were designed and synthesized by Shenzhen Huada Gene Co., Ltd. (Table II). PCR amplification reaction conditions: 94˚C for 5 min, followed by 35 cycles of 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 1 min, and 72˚C for 5 min. Primer sequences used in PCR reactions are shown in Table II PCR product was purified using Qiaquick column (Qiagen, Hilden, Germany). PCR products were digested with exonuclease I (New England Biolabs, Inc., Ipswich, MA, USA) and sequenced at a concentration of 50 ng/μl. Sequencing results were analyzed by 3730 gene analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The process was in accordance with the instructions of incision enzyme (New England Biolabs, Inc.) for digestion of the PCR products. RR fragment was 177 bp; RK fragment was 177 bp, which was cut into 107 bp and 70 bp; KK type fragment was 177 bp, and then cut into 107 bp and 70 bp. Electrophoresis in 2% agarose gel at 50 V voltage for 3 h. The product was dyed with acetethidine bromide. DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for PCR product sequencing analysis to confirm that the product was correct (Figs. 1 and 2).

Table III. Comparison of blood lipid level between the two groups.

Groups	n	TG (mmol/l)	TC (mmol/l)	HLD-C (mmol/l)	LDL-C (mmol/l)	ApoA-I (g/l)	ApoB (g/l)	Lp(a) (mg/l)
Control group	342	1.37±0.54	4.68±0.45	1.47±0.27	2.73±0.33	1.38±0.31	0.94±0.14	216.6±76.3
Observation group	112	1.72±0.65	4.66±0.38	1.18±0.22	2.76±0.28	1.27±0.27	0.87±0.16	222.4±72.5
t value		6.092	0.127	7.326	0.204	8.336	9.005	0.086
P-value		<0.05	>0.05	<0.05	>0.05	<0.05	<0.05	>0.05

Table IV. Comparison of ABCA1 R219K genotypes between the two groups.

		ABCA1 R219K genotype [n (%)]			Allele frequency (%)	
Groups	n	RR	RK	KK	R	K
Control group	342	(33.5)	(51.5)	(15.0)	56.4	43.6
Observation group	112	(28.2)	(54.6)	(17.2)	55.5	44.5
χ^2 value		0.156	0.163	0.285	0.204	0.216
P-value		>0.05	>0.05	>0.05	>0.05	>0.05

Table V. Effects of ABCA1 R219K genotypes on blood lipid level.

Types	TG (mmol/l)	TC (mmol/l)	HLD-C (mmol/l)	LDL-C (mmol/l)	ApoA-I (g/l)	ApoB (g/l)	Lp(a) (mg/l)
RR type (n=146)	1.41±0.87	4.72±0.76	1.32±0.27	2.71±0.42	1.36±0.27	0.84±0.12	213.6±80.5
RK type (n=234)	1.53±0.72	4.82±0.88	1.34±0.34	2.76±0.56	1.37±0.29	0.88±0.21	227.6±78.3
KK type (n=74)	1.46±0.68	4.75±0.76	1.41±0.65	2.82±0.52	1.41±0.32	0.86±0.25	225.7±71.6
F-value	2.654	1.054	1.658	2.287	1.892	1.634	1.927
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Statistical processing. Statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) software package. Most of the measurement data met the approximately normal distribution, while lipoprotein (a) [Lp(a)] met the skewed distribution, and it was tested after logarithmic transformation. The t-test was used for the intergroup comparisons of measurement data, and F-test was used for the comparisons among three groups; Chi-square test was used for categorical data.

Results

Comparison of general data between two groups. There were no significant differences in general data between two groups (P>0.05) (Table I).

Comparison of blood lipid level between the two groups. There were no significant differences in serum levels of TC, LDL-C and Lp(a) between the two groups (P>0.05). Levels of ApoB and TG in observation group were significantly higher than those in control group, but the levels of HDL-C and ApoA-I were significantly lower than those in control group (P<0.05) (Table III).

Comparison of ABCA1 R219K genotypes between the two groups. There were no significant differences in the RR, RK and KK frequencies and allele frequency of *ABCA1* R219K genotype between the two groups (P>0.05) (Table IV).

Effects of ABCA1 R219K genotypes on blood lipid level. The levels of HDL-C, TG, TC, LDL-C, ApoA-I, ApoB and Lp(a) showed no significant differences among different genotypes of *ABCA1* R219K (P>0.05) (Table V).

Discussion

ABCA1 is a type of common and highly conserved transmembrane protein that plays a key role in mediating the reverse transport of cholesterol (9-12). *ABCA1* R219K gene polymorphism has been proved to have effects on the blood lipids, but effects of R219K gene polymorphism on human blood lipid profile remains unknown. Most studies suggest that the TG

level is decreased and the HDL-C level is increased in K allele carriers (13-15). However, our data showed that there was no significant difference in HDL-C level between different R219K genotypes and control group.

Arteriosclerosis is a pathological basis of pathogenesis of cardiovascular and cerebrovascular diseases, as well as the main factor of the incidence of those diseases. Studies have shown that degree of arteriosclerosis is reduced in *ABCA1* R219K mutation carriers (16). In addition, in *ABCA1* R219K mutation carriers, cerebral infarction lesions can be easily repaired, and the incidence of coronary heart disease is also reduced. Some scholars (17,18) studied patients with ischemic stroke and found that R allele frequency in patients with cerebral infarction is lower than that in healthy population. However, further analysis found that *ABCA1* R219K gene polymorphism is not an independent risk factor for ischemic stroke. Some scholars (19) found that K allele frequency in *ABCA1* R219K has a protective effect on patients with coronary heart disease and ischemic stroke.

As a common type of ischemic stroke, LI can be induced by various factors, such as hypertension and cerebral arteriosclerosis (20-23). Correlation between LI complicated with arteriosclerosis and *ABCA1* gene polymorphism remains unclear. *ABCA1* R219K gene polymorphism can increase the beneficial blood lipid profile and reduce the severity of arteriosclerosis. At the same time, atherosclerosis can promote the occurrence and development of hypertension (24-28). However, it was found in this study that *ABCA1* R219K polymorphism had no correlation with LI complicated with arteriosclerosis. This study is still challenged by the small sample size, and we only focused on one SNP type only, which may affect the results. Future studies with larger number of samples are still needed to confirm the conclusion.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YX and ZL conceived and designed the study, collected, analyzed and interpreted the patient data. YX drafted the manuscript. ZL revised the manuscript critically for important intellectual content. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Ningbo First Hospital (Ningbo, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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